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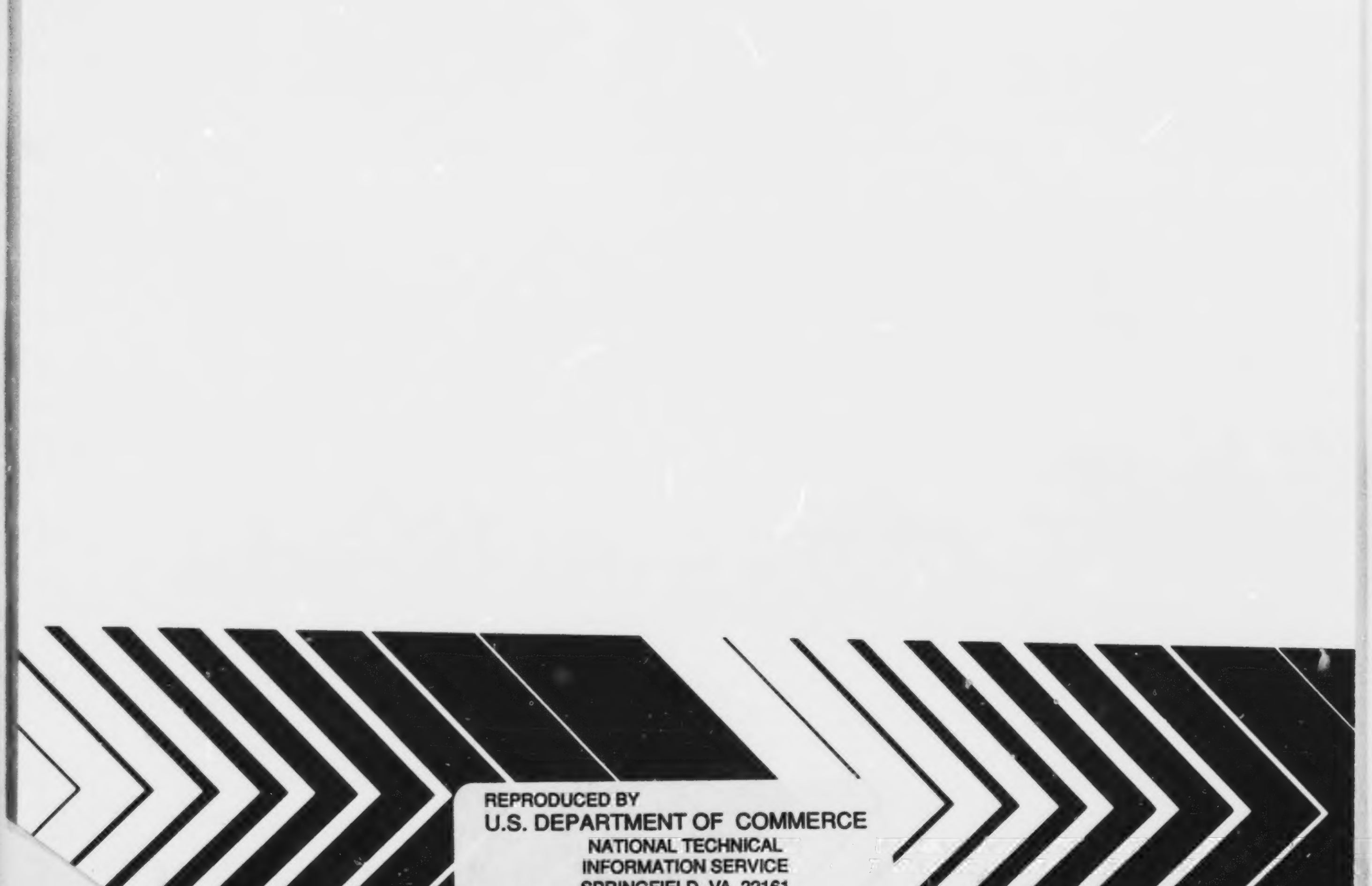

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MACROINVERTEBRATE FIELD AND LABORATORY METHODS FOR EVALUATING THE BIOLOGICAL INTEGRITY OF SURFACE WATERS

by

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FOREWORD

Environmental measurements are required to determine the chemical and biological quality of drinking water, surface waters, ground waters, waste waters, sediments, sludges, and solid waste. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate methods to identify and measure the concentration of chemical pollutants.
- Identify and quantitate the occurrence of viruses, bacteria, other human pathogens and indicator organisms.
- Perform ecological assessments and measure the toxicity of pollutants to representative species of aquatic organisms and determine the effects of pollution on communities of indigenous freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Develop and operate a quality assurance program to support achievement of data quality objectives for environmental measurements.

This manual describes guidelines and standardized procedures for the use of macroinvertebrates in evaluating the biological integrity of surface waters. It was developed to provide biomonitoring programs with the most recent benthic invertebrate methods for measuring the status and trends of environmental pollution on freshwater, estuarine, and marine macroinvertebrates in field and laboratory studies. These studies are carried out to assess water quality criteria for the recognized beneficial uses of water and to monitor surface water quality.

Thomas A. Clark
Director
Environmental Monitoring Systems
Laboratory - Cincinnati

PREFACE

The Aquatic Biology Branch, Quality Assurance Research Division, Environmental Monitoring Systems Laboratory - Cincinnati is responsible for the development, evaluation and standardization of methods for the collection of biological field and laboratory data by EPA regional, enforcement, and research programs engaged in inland, estuarine, and marine water quality and permit compliance monitoring, and other studies of the effects of impacts on aquatic organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish. The program addresses methods for sample collection; sample preparation; organism identification and enumeration; the measurement of biomass and metabolic rates; the bioaccumulation and pathology of toxic substances; bioassay; biomarkers; the computerization, analysis, and interpretation of biological data; and ecological assessments. Biological methods recommended for use in the U.S. Environmental Protection Agency are included in this manual: "Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters."

This document provides macroinvertebrate methods for evaluating the biological integrity of fresh, estuarine, and marine waters. The subjects covered include selection of sample sites, qualitative and quantitative sampling methods, sample processing, data analysis techniques, quality assurance and quality control procedures, safety and health recommendations, taxonomic bibliography, and the pollution tolerance of selected macroinvertebrate species.

The manual is a revision and enlargement of the chapter on macroinvertebrate methods originally published in the document, "Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents," Environmental Monitoring Series, USEPA, 1973, EPA-670/4-73-001, and was developed in the Aquatic Biology Branch, Environmental Monitoring Systems Laboratory - Cincinnati to provide biomonitoring programs with current methods for assessing point and non-point sources of impacts, status and trends water quality monitoring.

ABSTRACT

This manual describes guidelines and standardized procedures for using benthic macroinvertebrates in evaluating the biological integrity of surface waters. Included are sections on quality assurance and quality control procedures, safety and health recommendations, selection of sampling stations, sampling methods, sample processing, data evaluation, and an extensive taxonomic bibliography of the benthic macroinvertebrate groups. Supplementary information on the pollution tolerance of selected species, examples of macroinvertebrate bench sheets and macroinvertebrate data summary sheets, and a list of equipment and supplies for conducting biomonitoring studies are provided in the Appendices.

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SECTION 1

INTRODUCTION

1.1 Benthic invertebrates comprise a heterogenous assemblage of animal groups (taxa) that inhabit the sediment or live on or in other bottom substrates in the aquatic environment. They vary in size from forms small and difficult to see without magnification to other individuals large enough to see without difficulty.

1.2 The benthic invertebrates that are large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings) and live at least part of their life cycles within or upon available substrates in a body of water or water transport system are defined as **macroinvertebrates**. If a more representative sample of the benthos such as chironomids and other small forms (e.g., naidid and tubificid oligochaetes or aquatic worms) is desired, a U.S. Standard No. 60 sieve (60 meshes per inch, 0.250 mm openings) may be used.

1.2.1 Benthos (n.), Benthic (adj.)--the community of organisms living in or on the bottom or other substrate in an aquatic environment.

1.2.2 Benthic invertebrate--an invertebrate of the benthos.

1.2.3 Habitat--the place where an organism lives; for example mud, gravel, rocks, shoreline, vegetation, twigs, leaf packs, riffle/run, pool, etc.

1.2.4 Microhabitat--a smaller and more restricted area in a habitat; the immediate environment of the organism.

1.3 The standard opening for estuarine and marine benthic animals is also U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings), and new benthic programs should use the No. 30 sieve for collecting these animals. To accommodate some historical data bases, a 1.0 mm screen, U.S. Standard No. 18 sieve may be used.

1.4 Any available substrate may provide suitable habitat for benthos, including bottom sediments, submerged logs, debris, pilings, pipes, conduits, vascular aquatic plants, root masses, filamentous algae, etc. The major taxonomic groups of freshwater macroinvertebrates include the insects, annelids, mollusks, flatworms, and crustaceans. The major invertebrate groups in estuarine and marine water are the mollusks, annelids, crustaceans, roundworms, cnidarians (coelenterates), sponges, bryozoans, and echinoderms.

1.5 The macroinvertebrates are important members of food webs, and their well-being is reflected in the well-being of the higher forms such as fish. Many invertebrates, such as the marine and freshwater shellfish (clams and mussels), are important commercial and recreational species. Some, such as mosquitoes, black flies, biting midges, leeches, Asiatic clams, and zebra mussels, are of considerable public health significance or are considered pests. Many forms are important for digesting organic material and recycling nutrients.

1.6 Benthic macroinvertebrates are frequently used as environmental indicators of biological integrity because they are found in most aquatic habitats. They are of a size that makes them easily collected. They can be used to describe the water quality conditions or health of the ecosystem components and to identify causes of impaired conditions.

1.6.1 A community of macroinvertebrates in an aquatic lentic or lotic ecosystem is very sensitive to stress; and, thus, its characteristics serve as a useful tool for detecting environmental perturbation resulting from introduced point and non-point sources of pollution. Because of the limited mobility of these benthic organisms and because many species have life cycles of a year or more, their characteristics are a function of conditions during the recent past, including reactions to infrequently discharged pollutants that would be difficult to detect by periodic chemical sampling.

1.6.2 Macroinvertebrates show responses to a wide array of potential pollutants (agricultural, domestic, industrial, mining, etc.), including those with synergistic or antagonistic effects that adversely affect the physiological, biochemical, and reproductive functions of the species. The analysis of changes in the makeup of different aquatic communities is one way to detect water quality problems. Knowledge of changes in the community structure (abundance and composition) and function (see Section 1.7) of benthic macroinvertebrates helps to indicate water quality status and trends in the aquatic environment. Also the regular sampling of macroinvertebrates can be used to document both spatial and temporal changes in the biological integrity of surface waters. Different types of environmental stress will often produce different macroinvertebrate communities.

1.6.3 In addition, because of the phenomenon of "biological magnification" and relatively long-term retention of toxic substances by benthic organisms, toxic materials such as metals, pesticides, radioactive materials, which are only periodically discharged into the environment or which are present at undetectable levels in the water or sediment, may be detected by chemical analyses of selected components of the macroinvertebrate community.

1.7 Individuals or groups of macroinvertebrates can be separated into trophic levels, such as herbivores, omnivores, or carnivores and, in stream ecosystems, functional feeding relationships (Cummins, 1973, 1974, 1975; Cummins and Klug, 1979; Cummins et al., 1984; Cummins and Wilzbach, 1985). In a well-balanced system, all three types will likely be present. They include deposit and detritus feeders, collectors, shredders, grazers or scrapers, parasites, scavengers, and predators.

1.8 In most biomonitoring studies, identification at, or near the species level will be required to determine water quality conditions (Resh and Unzicker, 1975). Tolerant species (Appendix A) will usually become dominant only in polluted waters.

1.9 In pollution-oriented studies of macroinvertebrate communities, there are basically three sampling approaches--qualitative, semi-quantitative, and quantitative--that may be utilized singly or in combination. These sampling approaches are used to link ecosystem endpoints to stresses (e.g., physical habitat alterations, inert solids, eutrophication, organic enrichment, thermal disruptions, ambient toxic wastes, and cumulative impacts) measured by bioindicator methods and techniques. See Section 5, Sampling Methods and Section 7, Data Evaluation.

1.10 During studies of water quality accommodations should be made for stream size, geographic location, and seasonality (Lenat, 1983). Also, flow conditions are related to the relative impact due to point and nonpoint sources of pollution. High flow usually increases the impact of nonpoint sources, while it reduces the impact of point sources. In streams with low flow, the reverse is often true. In addition, the presence, distribution, and abundance of aquatic macroinvertebrates, especially aquatic insects, may be subject to wide seasonal variations (Hilsenhoff, 1988). Thus, when conducting comparative studies, the investigator must be careful to avoid the confounding effects of these seasonal changes. Seasonal variations are particularly important in freshwater habitats dominated by aquatic insects having several life stages, not all of which are aquatic.

1.11 The design of macroinvertebrate studies should be based upon study goals and data quality objectives (DQOs) (See Section 2, Quality Assurance and Quality Control). To supplement the material contained in this manual, a number of basic references should be reviewed or available to investigators of the macroinvertebrate communities, particularly to investigators engaged in aquatic water quality and pollution studies. These include Armitage (1978), Benke, Gillespie, and Van Arsdall (1984), Brinkhurst (1974), Cairns and Dickson (1973), Cummins (1966, 1973, 1974, 1975), Cummins and Klug (1979), Cummins et al. (1984), Cummins and Wilzbach (1985), Edmondson and Winberg (1971), Elliott (1977), Goodnight and Whitley (1960), Hart and Fuller (1974), Hellawell (1978, 1986), Hilsenhoff (1977), Howmiller and Scott (1977), Hynes (1960, 1970), Holme and McIntyre (1971), Hulings and Gray (1971), Lenat (1983), Lind (1974), Merritt and Cummins (1984), Mason (1981), Metcalfe (1989), Milbrink (1983), Meyer (1990), Neuswanger, Taylor, and Regnolds (1982), Pennak (1989), Posey (1990), Resh (1979), Resh and Rosenberg (1984), Resh and Unzicker (1975), Reynoldson et al. (1989), Ward and Stanford (1979), Warren (1971), Waters (1977), Welch (1948), Welch (1980), and Winner et al. (1975).

1.12 This manual was composed to assist biologists and managers in USEPA and other Federal, state, and private water monitoring organizations in the use of macroinvertebrates for evaluating the biological integrity of surface waters. The manual contains laboratory and field methods that will aid in the monitoring, detection, and bioassessment of surface waters and the effects of environmental stress on macroinvertebrate communities. It will also facilitate the expansion of our knowledge of the ecological requirements of macroinvertebrate species in fresh, estuarine, and marine habitats. The manual includes sections on quality assurance and quality control, safety and health, sampling site selection, sampling methods and techniques, sample processing, data evaluation, and a taxonomic bibliography, containing the current taxonomy used for identifying the macroinvertebrates of North America. Information on the pollution tolerance of selected species and examples of bench and data summary sheets are provided in the Appendices.

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SECTION 2

QUALITY ASSURANCE AND QUALITY CONTROL

2.1 Introduction

2.1.1 A strong quality assurance (QA) program and effective quality control (QC) procedures are needed for operating an adequate macroinvertebrate bioassessment or monitoring laboratory to ensure that all data produced are valid and of known quality. The term "quality assurance" refers to the quality control functions and involves the totally integrated program for ensuring the reliability of monitoring data; the term "quality control" refers to the routine application and procedures for obtaining prescribed standards of performance and for controlling the measurement process (USEPA, 1978). Quality assurance programs have two primary functions in a macroinvertebrate laboratory. First, the program should continually monitor the reliability of the data generated to determine the accuracy, precision, completeness, comparability, and representativeness of the data. The second function is the control of the quality of the data so as to meet the requirements for reliability that the program demands. Quality assurance and control must be a continuous process that includes all aspects of the program, including field collection and preservation, sample processing, and data analysis; otherwise the data generated may not be reliable and useful for decision making and the results will be of little use in establishing the biological integrity of the water body under study. In order to support the operation of a consistent plan, the persons responsible for QA should consult the EPA Quality Assurance manual (USEPA, 1984a). All EPA QA programs should be based on USEPA order 5360.1 (USEPA, 1984b) which describes the policy, objectives and responsibilities of all USEPA program and regional offices.

2.1.2 Components of the QA program (USEPA, 1979) should include the following:

2.1.2.1 Collection, preservation and analysis of all samples should follow approved methodology.

2.1.2.2 Sampling equipment, flow measuring devices, and other measuring instruments such as pH, DO, and conductivity meters should be calibrated according to manufacturer's instructions, and documented.

2.1.2.3 Assurance that representative samples are collected (See Section 4, Selection of Sampling Sites).

2.1.2.4 Determination of precision of sampling and analysis procedures.

2.1.2.5 Use of replication in all phases of the sampling and analysis program.

2.1.2.6 Participation in interlaboratory investigations and use of quality control samples.

2.1.2.7 Accurate and timely recording, maintenance, and storage of data in a log book, computer, or other data storage and retrieval system.

2.2 Data Quality Objectives (DQOs)

2.2.1 A full assessment of the data quality needed to meet the study objectives should be made prior to preparation and implementation of the QA plan. Data quality is a measure or description of the type and amount of error associated with a set of data. Determination of data quality is accomplished through the development of data quality objectives (DQOs), which are statements of the level of uncertainty a decision-maker is willing to accept or the quality of the data needed to support a specific environmental decision or action. Both qualitative and quantitative descriptors of data quality must be considered in order to determine whether data are appropriate for a particular application. Data quality objectives are target values for data quality and are not necessarily criteria for the acceptance or rejection of data.

2.2.2 Data quality objectives are developed in three stages. During the first stage, the decision-maker determines what information is needed, reasons for the need, how the information will be used, and specifies time and resource constraints. The second stage involves the technical staff and decision-maker interacting to establish a detailed and clarified specification of the problem, how the information will be used, any constraints imposed on the data collection, and what limitations of the information will be acceptable. The third stage involves the analysis of possible approaches to collection and analysis of the data and a determination of the quality of the data that can be expected to result from each approach. The best approach is selected based on the criteria agreed upon in the second stage. It may be necessary to modify the objectives of the study during the development of these DQOs. Details for developing DQOs are described in two U.S. Environmental Protection Agency documents (USEPA, 1984c and 1986) available from the Quality Assurance Management Staff, Office of Research and Development, Washington, DC 20460.

2.2.3 After the final DQOs are established, the detailed project QA plan should be finalized stating specific quantitative and qualitative data quality goals and QC procedures that will be used to control and characterize error (USEPA, 1980). The goals based on the DQOs will be the criteria for measuring the success of the QA program.

2.2.4 The Quality Assurance Management Staff, Office of Modeling, Monitoring Systems, and Quality Assurance, is responsible for providing guidance for the inclusion of DQOs in quality assurance program and project plans, and for providing guidance to the regions on the application of the DQOs development process. The EPA regional offices are responsible for ensuring that state QA program and project plans conform with grant requirements specified in 40 CFR Part 30, and for assisting the states in developing DQOs requirements that meet state needs.

2.2.5 Regional and state laboratories or monitoring personnel in need of assistance in preparing Quality Assurance Project Plans or development of DQOs for bioassessment projects can contact personnel of the Aquatic Biology Branch in the Quality Assurance Research Division, Environmental Monitoring Systems Laboratory-Cincinnati, for assistance (FTS 684-8114 or COML 513-533-8114, FAX FTS 684-8181 or COML 513-533-8181).

2.3 Facilities And Equipment

2.3.1 Laboratory and field facilities and utility services must be in place and operating consistent with their designed purposes so that quality environmental data may be generated and processed in an efficient and cost-effective manner. Suitability of the facilities for the execution of both the technical and QA aspects of the study should be assessed prior to initiation of the study. Adequate space, lighting, temperature, noise levels, and humidity should be provided. Satisfactory safety and health maintenance features must also be provided (see Section 3, Safety and Health).

2.3.2 Equipment and supplies necessary to adequately collect, preserve and process biological samples must be available and in good operating condition. See Appendix E for a list of recommended equipment and supplies.

2.3.2 To ensure data of consistently high quality, a plan of routine inspection and preventive maintenance should be developed for all facilities and equipment. All inspections, calibrations, and maintenance must be documented in individually bound notebooks. This documentation should include detailed descriptions of all calibrations performed, adjustments made, and parts replaced and each entry should be signed and dated.

2.3.3 Taxonomists and aquatic biologists who are capable of identifying organisms are expected to have at their disposal adequate taxonomic references to perform the level of identification required. See Section 8, Taxonomic Bibliography, for a list of selected taxonomic references. Aquatic biologists should check this list and obtain those references that will be needed for the identification of specimens to the lowest taxonomic level possible.

2.3.4 Representative specimens of all taxa identified should be verified by a specialist who is a recognized authority in that particular taxonomic group. These specimens should be properly labeled as reference or voucher specimens, including the name of the verifying authority, permanently preserved, and stored in the laboratory for future reference.

2.4 Calibration, Documentation, and Record Keeping

2.4.1 Quality assurance plans should contain mechanisms for demonstrating the reproducibility of each measuring process. Regular calibration of instruments, proper documentation, and permanent record keeping are essential aspects of such plans.

2.4.2 Each measuring device must be calibrated before each use according to the manufacturer's instructions, and routine checks using National Institute of Standards and Technology, or other standards of known accuracy, should be made to demonstrate that variables are within predetermined acceptance limits. Permanent records giving dates and details of these calibrations and checks must be kept. Documentation is necessary to identify each specific measuring device, where and when it is used, what maintenance was performed, and the dates and steps used in instrument calibration. Each sample collected should also be documented by assigning a unique identification number and label (See Section 6, Sample Processing). Data should be documented to allow complete reconstruction, from initial field record through data storage system retrieval.

2.4.3 Whenever samples are collected to be used as evidence in a court of law, it is imperative that laboratories and field operations follow written chain-of-custody procedures for collecting, transferring, storing, analyzing, and disposing of the samples. The primary objective of chain-of-custody procedures is to create written record which can be used to trace the possession of the sample from the moment of collection through the introduction of the analytical data into evidence. Explicit procedures must be followed to maintain the documentation necessary to satisfy legal requirements. All survey participants should receive a copy of the study plan and be knowledgeable of its contents prior to implementing the field work. A presurvey briefing should be held to reappraise all participants of the survey objectives and chain-of-custody procedures. After all chain-of-custody samples are collected, a debriefing should be held in the field to check adherence to chain-of-custody procedures. Chain-of-custody procedures are detailed in three USEPA manuals (USEPA, 1974, 1982, and 1990).

2.4.4 Field and laboratory personnel should keep complete and permanent records of all conditions and activities that apply to each individually numbered sample sufficient to satisfy legal requirements for any potential enforcement or judicial proceedings. All field and laboratory data sheets should be dated and signed by the sampler and analyst, respectively. Notebooks, data sheets, and all other records that may be needed to document the integrity of the data should be kept permanently filed in a safe and fireproof place.

2.5 Qualifications and Training

2.5.1 All personnel need to have adequate education, training, and experience in the areas of their technical expertise and in QA to fulfill their designated responsibilities. Because no formal academic programs in research QA exist, most QA experience will have to be acquired through on-the-job training.

2.5.2 At least one professional biologist with training and experience in biological sampling methods and macroinvertebrate identification should be on the staff and should be personally involved in the field work as well as the laboratory analysis of the samples. Statistical expertise should be readily available and consulted during every phase of the project.

2.5.3 Management should periodically assess the training needs of all personnel engaged in QA and recommend and support their participation in appropriate and relevant seminars, training courses, and professional meetings. Biologists and technicians should be expected to participate regularly in evaluation and/or certification programs where appropriate.

2.5.4 The laboratory should have on file an up-to-date resume for each person who is responsible for the analysis, evaluation and reporting of biological data.

2.6 Standard Operating Procedures (SOPs)

2.6.1 Each laboratory must define the precise methods to be used during each step of the sample collection, analysis, and data evaluation process. These written procedures become the standard operating procedures (SOPs) describing the operation of the laboratory. Standard operating procedures for a macroinvertebrate laboratory should describe in stepwise language, easily understood by the potential user, the sampling methodology, details of preservation and labeling the samples, use of taxonomic keys, use and calibration of measuring instruments, replication and QC requirements, sample custody and handling procedures, and data evaluation and handling. The SOPs should include a listing of the taxonomic keys and references that should be used for each level of identification required and for each taxonomic group. It should provide an outline of the steps to be taken to assure the quality of the data.

2.6.2 The SOPs should stress the need for the traceability of the samples. At a minimum it should specify that each sample be assigned a unique identification number and be properly labeled with the sample number, sampling location, and name of the collector. It should describe procedures to ensure that each sample collected, as accurately and precisely as possible, represents the community sampled.

2.6.3 The SOPs should be approved by the proper authority and should be easily accessible to personnel for referral.

2.6.4 The laboratory SOPs should be followed as closely as possible. Any deviations should be documented as to the reason for the deviation and any possible effect the deviation might have on the resulting data.

2.7 Literature Cited

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SECTION 3

SAFETY AND HEALTH

3.1 Introduction

3.1.1 Collection and analysis of benthic samples involve significant risks to personal safety and health. While safety is often not considered an integral part of benthic sampling routine, the biologist must be aware of unsafe working conditions, hazards connected with the operation of sampling gear, and other risks. Management should assign health and safety responsibilities and establish a program for training in safety, accident reporting, and medical and first aid treatment. Written safety policies should be available to all persons involved in the sampling and analysis of macroinvertebrate samples and this should include a copy of the USEPA (1986) safety manual.

3.2 General Precautions

3.2.1 Basic good housekeeping practice should be followed both in the field and in the laboratory. These practices should be aimed at protecting the staff from physical injury, preventing or reducing exposure to hazardous or toxic substances, avoiding interferences with laboratory operations, and producing valid data.

3.2.2 Operation of benthic sampling devices involves hazards that must be addressed by the person using the equipment. Some grab samplers (e.g., Ekman, Smith-McIntyre) have spring loaded cocking devices that can cause serious injury if not handled and operated carefully. Other grabs (e.g., Ponar) have safety locking pins that must be put in place to prevent injury. Persons using these devices should become familiar with the hazards involved and establish appropriate safety practices prior to using them.

3.2.3 Field personnel should known how to swim. Waders should always be worn with a belt to prevent them from filling with water in case of a fall. A life jacket at dangerous wading stations is advisable if one is not a strong swimmer because of the possibility of sliding into deep holes.

3.2.4 Many hazards lie out of sight in the bottoms of lakes, rivers and streams. Broken glass or sharp pieces of metal embedded in the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that may be absorbed through the skin or inhaled may also be present in the water or sediment.

3.2.5 Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators should be familiar with U.S. Coast Guard rules and regulations for safe boating contained in a pamphlet, "Federal Requirements for Recreational Boats," available from your local U.S. Coast Guard Director or Auxiliary or State Boating Official (U.S. Coast Guard, 1987).

3.2.6 Prior to a sampling trip, personnel should determine that all necessary equipment is in safe working condition and that the operators are properly trained to use the equipment.

3.2.7 Safety equipment and first aid supplies should be available in the laboratory and in the field at all times. A snake bite kit should be carried on all field trips in areas that may be infested with poisonous snakes. All motor vehicles and boats with motors should have fire extinguishers.

3.3 Safety Equipment and Facilities

3.3.1 Necessary and appropriate safety apparel such as waders, lab coats, gloves, safety glasses, and hard hats should be available.

3.3.2 First aid kits, fire extinguishers and blankets, safety showers, and emergency spill kits should be readily available in the laboratory at all times.

3.3.3 A properly installed and operating hood should be provided in the laboratory for use when working with volatile chemicals that may produce dangerous fumes.

3.3.4 Communication equipment should be available to field personnel and those working in mobile labs in remote areas for use in case of an emergency.

3.3.5 Facilities and supplies should be available for cleaning of exposed body parts that may have been contaminated by pollutants in the water. Soap and an adequate supply of clean water or ethyl alcohol may be suitable for this purpose.

3.4 Field and Laboratory Operations

3.4.1 At least two persons should be involved in all field collecting trips and no one should be left alone while in the field.

3.4.2 All surface waters should be considered potential health hazards due to toxic substances or pathogens and exposure to them should be minimized as much as possible. Exposed body parts should be cleaned immediately after contact with these waters.

3.4.3 All electrical equipment should bear the approval of Underwriters Laboratories and be properly grounded to protect against electric shock.

3.4.4 Staff training in basic first aid and cardio-pulmonary resuscitation is strongly recommended.

3.4.5 Before transporting grab sampling devices, be sure all safety lock pins are in place or transport them in the closed position. Read and follow all safety instructions provided by the manufacturer.

3.4.6 Use a winch for retrieving samples collected with heavy sampling devices such as the Ponar grab and use care in lifting heavy items to prevent back injury.

3.4.7 Heavy gloves should be used when hands are used to agitate the substrate during collection of square-foot type samples and when turning over rocks during hand picking.

3.4.8 Persons working in areas where poisonous snakes may be encountered should check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a poisonous snake. If local advice is not available and medical assistance is over an hour away, carry a snake bite kit and be familiar with its use. Any person allergic to bee stings or other insect bites should take proper precautions and have any needed medications handy.

3.4.9 Personnel dealing in field activities on a regular or infrequent basis should be in sound physical condition and have a physical exam annually in accordance with Regional or State Safety Officer's requirements.

3.4.10 Hypothermia--all field personnel should be familiar with the symptoms of hypothermia and know what to do in case symptoms should occur. Hypothermia can kill a person at temperataure much above freezing (up to 50°F) if he or she is exposed to wind and rain or otherwise becomes wet.

3.5 Disease Prevention

3.5.1 Because it is not known what pollutants may be present in surface waters and sediments, they should be considered potential health hazards and exposure to them kept to a minimum.

3.5.2 Personnel, who may be exposed to water known or suspected to contain human wastes, should be immunized against tetanus, hepatitis, typhoid fever, and polio.

3.6 Literature Cited

US Coast Guard. 1987. Federal requirements for recreational boats. U.S. Department of Transportation, United States Coast Guard, Washington, DC 20593.

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SECTION 4

SELECTION OF SAMPLING STATIONS

4.1 Introduction

4.1.1 The design of monitoring programs is one of the major sources of error or uncertainty in water quality data (Thornton et al., 1982). Proper selection of sampling sites (overall sampling areas) should be directed toward minimizing uncertainty or, at least, provide a means by which variability may be reduced.

4.1.2 If samples are taken at random over the whole stream, river or lake bottom, the sample sites may differ physically and species counts can be highly variable. A reasonable sample size would be expected to detect only a population density difference of more than 200% of the mean between two sites (Schwenneker and Hellenthal, 1984). If, however, the potential sampling areas are restricted to those of similar physical nature, this variability will be reduced so that differences of 50% or better can often be obtained. Mason et al. (1973) found that three artificial substrate replicate samples could be expected to give estimates within 20% of the true mean at the 95% confidence level.

4.1.3 Chutter and Noble (1966) studied the effects of sample site selection using the Surber square foot sampler and concluded that the closer the sampling site is defined the more reliable will be the sampling data in terms of a single species per square foot. Therefore, selection of sampling sites with similar substrate types (e.g., particle size), current velocity, depth and other physical characteristics will aid greatly in reducing variability.

4.1.4 Most organisms, even in a selected and defined habitat type, are not evenly distributed over the bottom of a waterbody so replicate samples will be needed to evaluate this variability (Cairns and Dickson, 1971). The crucial question is how many samples should be taken. The answer will depend on the purpose of the study, data quality objectives, physical characteristics of the sampling location, the type of sampler to be employed, and the time available. Mackey et al. (1984) considered four replicates in each distinctive environmental zone along the river to be adequate when using pond nets. A minimum of two replicate samples at each station are required when using drift nets (Lewis et al., 1975). Two (Mason et al., 1973) or three replicate samples are required for artificial substrate type samplers, and three replicate samples are the absolute minimum when using Surber and Hess type samplers (Needham and Usinger, 1956) or grab samplers (Lewis et al., 1982). In most cases collecting five replicate samples at each station will increase the statistical precision and accuracy. Additional replicate samples may be necessary to characterize the benthic community in some aquatic environments. The number of replicate samples needed should be determined during the reconnaissance or pilot study. The samples from various habitats should be processed and analyzed separately. The data can be aggregated later after individual samples are analyzed and tabulated, but potentially important comparisons among habitats are lost if samples are composited.

4.1.5 A sound sampling design requires substantial understanding of the organisms being sampled and the types and limitations of the sampling devices to be employed. Data reduction techniques also, should be included in the study plans. Knowledge of locations of possible sources of pollution as well as insight into the intensity of the expected effects of the environmental changes that may be occurring at the site are also of great value. Other factors that will need to be involved in proper selection of the sampling sites include objectives of the study, accessibility, flow and mixing characteristics of effluents, personnel and facilities available to conduct the study, and historical data from previous studies. The primary concern in designing a sampling scheme is to gain an accurate measurement with high precision with the least effort possible to optimize productivity of available person-hours (Downing, 1979).

4.2 Location of Sampling Stations (Sampling Locations Within Each Site)

4.2.1 After determining the specific data quality objectives of the study and defining clearly what information is needed, it is necessary to select specific reaches of the stream or areas of the lake to use as sampling sites. Reconnaissance of the waterway (pilot study) at this time, using the Rapid Bioassessment Protocol I (Plafkin et al., 1989) or similar techniques, is important. Note possible sources of pollution, access points, bottom types, flow characteristics, and other physical characteristics that will need to be considered in selecting the sampling sites. The results of the pilot study may be used to obtain estimates of variances needed to establish sample size. Other advantages of the pilot study are that it accomplishes a detailed reconnaissance and it provides the opportunity to obtain experience in the actual field situation where the final study will be made. Information obtained and difficulties encountered may often be used to avoid costly and needless expenditures during the full scale study. Although the number and location of sampling stations will vary with each individual study, the following basic rules modified from Cairns and Dickson (1971), if carefully followed, should result in a sound survey design.

4.2.1.1 Always have at least one reference station (control station) away from all possible discharge points to provide a basis for comparison between areas above and below the point of discharge. This station should be directly above the effluent discharge in streams or just outside the zone influenced by the discharge in lakes and estuaries. It is advisable to add a second reference station well above or outside the zone of influence. See Section 4.3, Selecting Control Stations.

4.2.1.2 Establish a station directly below the source of pollution in streams or at the point of discharge into lakes. If the discharge does not mix completely immediately on entering a stream, left-bank, midchannel, and right bank substations should be established.

4.2.1.3 Establish stations at various distances downstream from the discharge in streams or away from the discharge point in lakes. Effort should be made to space the collecting stations approximately exponentially farther apart going down stream from the pollution source to determine the extent of the recovery zone.

4.2.1.4 All sampling stations should be as ecologically similar as possible in order to compare the benthic fauna collected at these sites. Decreasing station similarity with regard to habitat parameters generally indicate decreasing station comparability. The ability to control or measure inherent natural variability will enhance the overall assessment of benthic community structure and function. Bottom substrate, depth, temperature, flow velocity, bank cover, and salinity, etc. should be similar at each site. Where stations cannot be located in areas of similar habitats it may be necessary to use artificial substrate samplers to collect the samples.

4.2.1.5 Sampling stations for macroinvertebrates should be close to the sites where sampling for chemical and physical analyses will be located.

4.2.1.6 Sampling stations should be located in areas where benthos is not influenced by atypical conditions, such as those created by bridges or dams unless effects of atypical conditions make up part of the study objectives. For instance, urbanized areas include these structures as typical, and, in some cases, may provide the best suitable habitat that is available.

4.2.1.7 Sampling stations should be located so that samples can be collected from all the stations in a study on approximately the same day. If samples are collected on different days, emergence of adults may occur at a later collection site resulting in erroneous conclusions.

4.2.1.8 The sampling stations should be in places that are easily accessible. Long hiking distances and steep banks should be avoided if at all possible. If a boat will be needed for sample collection, the station should be located near a boat dock or launch ramp. In some habitats, such as a large lake, estuary, or ocean, sampling stations will, of necessity, often be miles from the boat launch ramp. If artificial substrate samplers are being used, the possibility of vandalism should be taken into account when selecting stations for installing these sampling devices.

4.2.2 Sampling to assess the effects of non-point sources of pollution requires a number of stations along the stream in the impacted area. Samples should also be collected in the unimpacted upstream area and the downstream recovery zone of the impacted stream.

4.3 Selecting Control Stations

4.3.1 Selecting appropriate control stations is a critical step because the control condition is the best estimate of integrity available to the investigator. The control station must be at a representative site at which conditions adequately reflect or approximate the conditions of the water body being investigated. Four basic approaches available as estimates of control conditions are: (1) consult historical records, (2) use pristine or least disturbed areas, (3) use ecoregion reference sites, and (4) use computer simulation techniques to create a hypothetical benthic community as a reference station.

4.3.1.1 Historical records may be incomplete or nonexistent but, if available, can often provide valuable information on the status of previous conditions at the site. Usefulness of computer simulation techniques will depend on the quality and quantity of data available on the site in question. The most viable option most of the time is the use of least disturbed areas as controls in combination with the other three approaches.

4.3.1.2 The investigator, therefore, must look for the least impacted areas as close to the impacted area as possible or to an ecoregion reference station as the control site. The ecoregion reference station represents the best attainable conditions for all streams (or other water bodies) with similar physical characteristics for a given ecoregion (Plafkin et al., 1989). Ecoregions are geographic patterns of similarity among ecosystems, grouped on the basis of environmental variables such as climate, soil type, physiography and vegetation. From the data base that has been generated at the ecoregion reference station it would be theoretically possible to determine the expected aquatic community structure that would exist in the study area if not impacted (or in its pristine condition). If the ecoregion reference station or a station in an adjacent area is used as the control site, a second control station should be sampled in the least impacted area of the water body under study for comparison. Care must be taken because most navigable waterways have been altered by channelization, dredging, bridge building, etc.

4.4 Study Design

4.4.1 Once the sampling stations are chosen, the investigator will need to determine exactly where the samples will be collected at each station in order to determine the biological integrity of the aquatic community. Two types of sampling plans are discussed: 1) random sampling is used when quantitative data is needed, and 2) non-random sampling may be used to generate qualitative data or semi-quantitative data.

4.4.2 Random Sampling

4.4.2.1 In biological studies using the quantitative sampling approach, the exact location of sample collection (sampling units) and number of samples to be collected at each station must be selected with some known probability that a certain measure of precision will be obtained. Usually, random selection is the only feasible means of satisfying this criterion. Only by knowing the probability of selecting a specific sample can one extrapolate from the sample to the population in an objective way. The probability allows one to place a weight upon an observation in making an extrapolation to the population. There is no other quantifiable measure of how well the selected sample represents the population. Thus the study plan should include an appropriate effort to define the problem in such a way as to allow a person to estimate the parameter of interest using a sample of known probability called a random sample.

4.4.2.2 There is a fundamental distinction between a "haphazardly-selected" sample and a "randomly-selected" sample. The distinction is that a haphazardly-selected sample is one where there is no conscious bias, whereas a randomly-selected sample is one where there is consciously no bias. There is consciously no bias because the randomization is planned and, therefore, bias is planned out of the study. This is usually accomplished with the aid of a table of random numbers. A sample selected according to a plan that includes random selection of experimental units is the only sample validly called a random sample.

4.4.2.3 Quantitative sampling in biological field studies is most often aimed at explaining spatial distributions of population densities or of some parameter related to population densities and the measurement of rates of change which permit prediction of some future course of a biologically-related parameter. In these cases the sampling unit is a unit of space (volume, area). Even in cases where the sampling unit is not a unit of space, the problem may often be stated in such a manner that a unit of space may be used, so that random sampling may be more easily carried out.

4.4.2.4 It is not always a simple or straightforward matter to define sampling units, because of the dynamic nature of the hydrology of streams and living populations. Many aquatic organisms are mobile, and even rooted or sessile forms change with time, so that changes occurring during the study often make data interpretation difficult. Thus, the benefit to be derived from any attempt to consider such factors in the planning stage will be considerable.

4.4.2.5 Random sample selection is a subject apart from the selection of the study site. It is of use only after the study objectives have been defined, the type of measurements have been selected, and the number of samples has been determined. At this point, random sampling provides an objective means of obtaining information to achieve the objectives of the study.

4.4.2.6 One satisfactory method of random sample selection is to number the universe, or entire set of sampling units available, from which the sample will be selected. This could be accomplished by marking off equal distances on a line transect across the stream and numbering each mark consecutively or by dividing a section of a water body (the sampling station) into grids as in figure 4 and numbering each intercept. The total number of marks or intercepts is "N". Then from a table of random numbers select as many random numbers, n, as there are sampling units selected for the sample. Select a starting point in the table and read the numbers consecutively in any direction (across, diagonal, down, up). For example, if "N" is twenty, select only numbers less than or equal to 20, ignoring any number greater than "N" or any number that has already been selected. These numbers will be the numbers of the sampling units to be selected (Cummins, 1962).

4.4.2.7 If a random starting point is chosen along the transect to introduce randomness needed to guarantee freedom from bias and allow statistical inference and the samples are collected at points chosen systematically along the transect, the data collected could be considered quantitative. To avoid arbitrariness, randomization should also be employed in transect placement.

4.4.2.8 Simple Random Sampling is used when there is no reason to subdivide the population from which the sample is drawn. The sample is drawn such that every unit of the population (numbered section or grid) has an equal chance of being selected. This may be accomplished by using the random selection scheme already described. Because the spacial distribution of benthic communities is so closely related to physical factors such as substrate type, current velocity, depth, and salinity, a design using simple random sampling is seldom meaningful. Therefore, it is usually best to stratify the habitat on the basis of known physical habitat differences and select sampling units by an appropriate randomization procedure in each habitat type; a procedure known as stratified random sampling.

4.4.2.9 Stratified Random Sampling is usually the preferred sampling design because of a resulting increase in precision. If any knowledge of the expected size or variation of the observations is available, it can often be used as a guide in subdividing the population (potential sampling points or units) into subpopulations (strata) (Gaufin *et al*., 1956). Information obtained during the pilot study will be useful in determining what strata to sample. The pilot study planning should be done carefully, perhaps stratifying based upon suspected variability in community structure. To maximize precision, strata should be constructed such that the observations are alike within strata and different among strata. In practice, the information used to form strata will usually be from previously obtained data or the pilot study. In aquatic field situations, stratification may be based upon bottom type, depth, isotherms, and numerous other variables suspected of being correlated with the characteristic of interest.

4.4.2.10 Stratification may also be done on other bases such as convenience or administrative imperative, but except where these correspond with criteria which minimize the variation within strata, no gain in precision may be expected.

4.4.2.11 Number of strata - In aquatic biological field studies, the use of knowledge of biological cause-and-effect may help define reasonable strata (e.g., thermoclines, sediment types, etc., may markedly affect the organisms so that the environmental features may be the obvious choice for the strata divisions). Where a gradient is suspected and where stratification is based on a factor correlated to an unknown degree with the characteristic of interest, the answer to the question of how many strata to form and where to locate their boundaries is not clear. Usually as many strata are selected as may be needed to meet the data quality objectives of the study. In practice, gains in efficiency with increasing stratification usually become negligible after only a few divisions unless the characteristic used as the basis of stratification is very highly correlated with the characteristic of interest.

4.4.2.12 For many quantitative studies, it is often necessary in the interest of economy and efficiency and within the limitations of the available gear, to sample primarily at sites having substrates which normally support the most abundant and varied fauna, and devote a minimum effort to those substrates supporting little or no life. For instance, in many large, swiftly flowing rivers of the midwest and southeast, the areas of "scour" with a substrate of shifting sand or hardpan may be almost devoid of macroinvertebrates; sampling effort may be reduced there in favor of the more productive areas of "deposition" on the inside of bends or in the vicinity of obstructions. Just the opposite situation may occur in many of the swiftly-flowing upland streams, where most of the effort may be devoted to sampling the productive rubble and gravel riffle areas instead of the pools.

4.4.2.13 When the location of sampling stations and placement of the samplers at these stations are done in a non-random manner, as is often done in practice, the sample is best considered a semi-quantitative sample even though a quantitative sampling device is used in the study.

4.4.3 Systematic Sampling

4.4.3.1 If quantitative data are not needed, some type of systematic sampling is generally employed for synoptic surveys and reconnaissance studies. Line transects established at discrete intervals across a river or stream and sampled at quarter points, or more frequent intervals, are a form of systematic sampling (Fig. 1). Use of this type of sampling assures an adequate cross section while maintaining relative ease of sampling. In lakes, reservoirs, wetlands, and estuaries, transects may be established along the short or long axis or may radiate out from a source of pollution (Fig. 2). The method of placement of the transect should be given a great deal of thought so that sampling stations will be as representative as possible. The confounding effects of changes in physical characteristics of the environment along the transect must be fully recognized and accounted for. A topographical map with fixed bench marks, a surveyor's sighting instrument mounted on a tripod, and surveying stakes marked off in centimeters are useful for establishing a line transect. The sampling points should be marked so that the fixed stations can be visited during each sampling visit. These fixed stations can be marked on a rope extended between poles on each side of a stream or buoys can be attached to weights on the bottom.

4.4.3.2 In lakes, reservoirs and estuaries the stations may be marked by use of sighting stakes or dabs of paint on rocks established on the shore. Two sighting lines should be established for each station so that they intersect at the fixed site (Fig. 3).

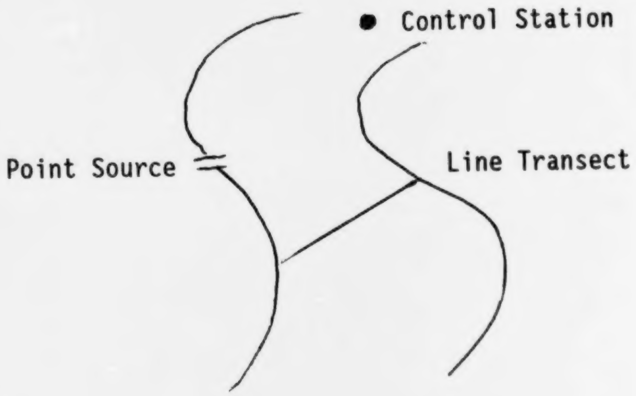
Control Station
Point Source
Line Transect

Figure 1. Example of transect sampling scheme in rivers and streams.

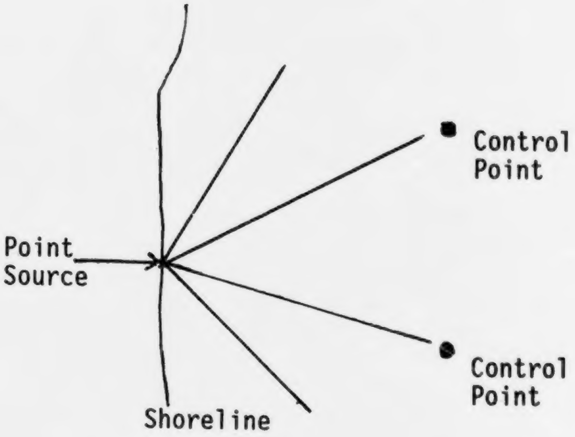
Control Point
Point Source
Control Point
Shoreline

Figure 2. Example of transect sampling scheme in lakes, reservoirs, and coastal waters.

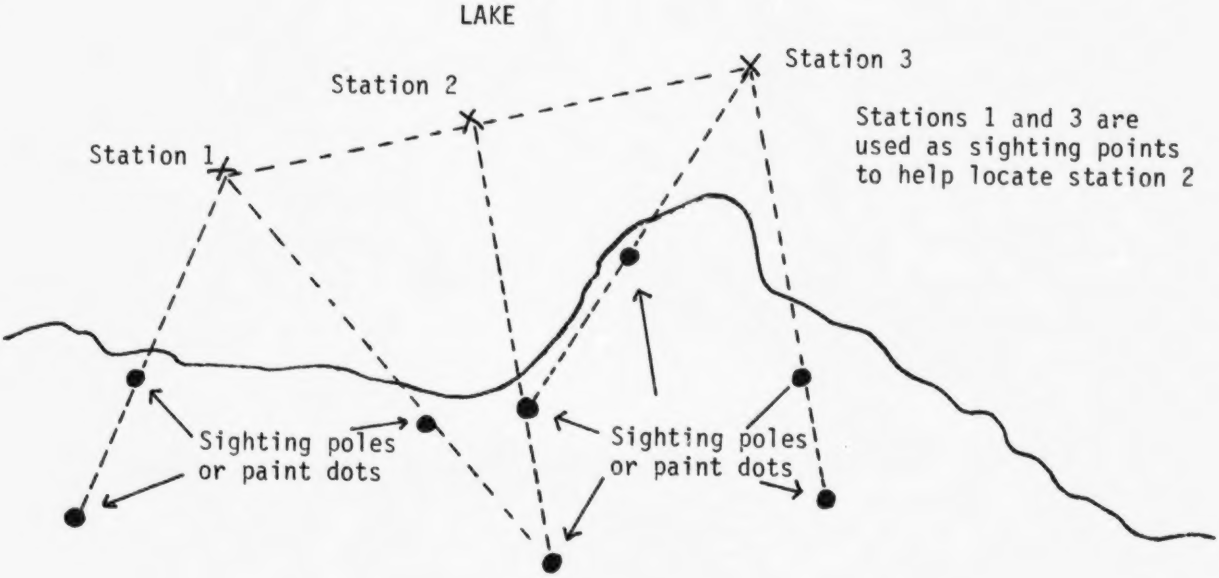
LAKE
Station 3
Station 2
Station 1
Stations 1 and 3 are used as sighting points to help locate station 2
Sighting poles or paint dots
Sighting poles or paint dots

Figure 3. Illustration of how sighting lines are used to locate fixed sampling locations in lakes, reservoirs, or estuaries.

4.4.3.3 Two other methods of locating stations on large water bodies are the Loran-C and Navstar/GPS methods of sighting longitude and latitude (USEPA, 1987).

4.4.3.4 Loran is an acronym for long range navigation. It is a pulsed low-frequency electronic navigation system that operates at 90 to 110 KHz in the hyperbolic mode. Loran-C has a nominal absolute accuracy of 185-460 meters over short distances using ground waves, whereas repeatable accuracy varies from 15-90 meters. Loran-C is frequently used for coastal monitoring programs, however, it can be used up to 160 Km inland if overland transmission of signals is used. User capability is unlimited.

4.4.3.5 Navstar/GPS is an acronym for Navstar Global Positioning System (GPS). It is a second generation satellite navigation system currently under development by the U.S. Department of Defense. Its purpose is to provide precise, continuous, worldwide, all-weather, three-dimensional navigation for land, sea, and air applications. More information on these and other systems can be found in "Evaluation of Survey Positioning

Methods for Nearshore Marine and Estuarine Waters" (USEPA, 1987).

4.4.3.6 Instead of line transects, the investigator may employ the grid sampling scheme in rivers lakes, reservoirs, wetlands, and estuaries as another type of systematic sampling (Fig. 4). Grid sampling may be either random or non-random depending on the method of choosing the sampling points within the grid as discussed above for transect sampling (See section 4.4.2.6).

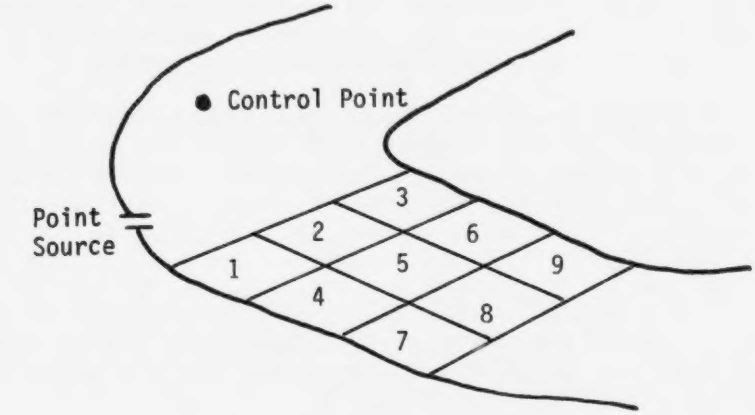

Figure 4. Example of grid sampling scheme in rivers.

4.4.3.7 In another form of systematic sampling, the investigator, using a variety of gear, consciously selects and intensively samples all recognizable habitat types. Such a non-random sampling plan may be used for collecting qualitative data. Non-random sampling is often employed during the reconnaissance phase of the study to gain a general idea of the type of benthic organisms that will be sampled during the main phase of the study. Use of kick nets in riffle areas and hand picking from rocks in pool areas are typical collection methods employed during this phase of the sampling program. These non-random sampling methods are also commonly used in rapid bioassessment studies (Plafkin et al., 1989).

4.4.3.8 In conducting synoptic surveys or other qualitative studies and taking into account the limitations of available sampling devices, sampling stations should be selected to include all substrate types. If these qualitative samples are to be used for determining the effects of pollutants where the pollutant does not have a direct effect on the substrate, the investigator must bear in mind that only the fauna from sites having similar substrates in terms of organic content, particle size, vegetative cover, and detritus will provide valid data for comparison.

4.5 Consideration of Abiotic Factors

4.5.1 Regardless of the method used to select the sampling unit, the biologist must consider and account for those natural environmental variations that may affect the distribution of organisms in the waterbody under investigation. Among the more important environmental variables in freshwater habitats are substrate type and stability, gradient, current velocity, flow rate, water depth (spates and drought in lotic waters), light and temperature regimes, and water quality characteristics such as dissolved oxygen, turbidity, acidity, hardness, alkalinity, sulfates, and nutrient concentration. In mountain ranges the elevation is an important consideration because it affects water temperature and other stream characteristics. In estuaries, additional variables that must be accounted for are the salinity gradient and tidal cycles.

4.5.2 Substrate Type is one of the most important factors for controlling the characteristics of the community of macroinvertebrates found at a given location in a body of water (Scott, 1958). Over a period of time, the natural substrates may be greatly altered by the discharge of particulate mineral or organic matter, and the location and expanse of various substrate types (silt, sand, gravel, etc.) may change because of normal variations in hydrologic factors such as current velocity and stream flow. The biologist, therefore, must be cognizant of changes in the nature and properties of the substrate which may provide clues on the quality and quantity of pollutants and other factors which affect the normal distribution of the benthic fauna.

4.5.2.1 Where the pollutant has a direct effect on the characteristics of the substrate, the effects of these changes may be inseparable from the effects of changes in water quality. Where substrate has deteriorated, faunal effects may be so obvious that extensive sampling may not be required and special attention should be given to the physical and/or chemical characterization of the deposits.

4.5.2.2 Because of the importance of substrate (in terms of both organic content and particle size) in macroinvertebrate studies, it is suggested that one or more unsieved substrate samples be collected from each station for use in conducting an analysis of substrate characteristics.

4.5.2.3 The mineral and organic matter content of the stream, lake, or estuary bottom at each sampling station should be classified and recorded on suitable forms, on a percentage basis, using the categories shown in Table 1, which should be applicable to most situations with only slight modification.

4.5.2.4 It is often desirable to further evaluate the inorganic components of the substrate by conducting a wet and dry particle size analysis in the laboratory. This analysis should be conducted on replicate samples from each sampling site with the use of standard sieves following the modified Wentworth classification shown in Table 2. Methods for separating the coarse fractions are given in Welch (1948). The silt-clay fraction may be considered "silt" if it is of a fine, loose consistency upon drying, and "clay" if it is of a sticky consistency forming hard clumps on drying (Lewis et al., 1982). If it is desirable to further separate the silt-clay fraction, a Coulter counter as described by Walker et al. (1974) is recommended.

TABLE 1. CATEGORIES FOR FIELD EVALUATION OF SUBSTRATE CHARACTERISTICS*

Type	Size or characteristic
Inorganic Components	
Bed rock or solid rock	
Boulders	>256 mm (10 in.) in diameter
Rubble/cobble	64 to 256 mm in diameter
Gravel	2 to 64 mm in diameter
Sand	0.06 to 2.0 mm in diameter
Silt	<0.06 mm in diameter, of a loose consistency easily disturbed
Clay-Marl/hard pan	<0.004 mm in diameter, of a sticky consistency not easily disturbed, slick feeling when rubbed between fingers
Organic Components	
Detritus	Wood, sticks, and other undecayed coarse plant materials
Peat	Variously decomposed, green to brown, plant remains
Muck	Completely decomposed, black, fine organic matter

*Modified from Roelofs, 1944.

4.5.2.5 Analysis of Organic Content - The organic content may be determined by drying and ashing a weighed amount of a representative sample of the sediment.

4.5.2.6 Dry weight is determined by weighing the sample in a tared porcelain crucible, drying in an oven at 105 degrees C to a constant weight (24 hours), and weighing.

4.5.2.7 Ash-free weight is determined after the dry weight is done. Place the same crucible with the dried sample in a muffle furnace at 500 degrees C for one hour. Cool, rewet the ash with distilled water, and bring to constant weight (about 24 hours) at 105 degrees C. The ash is wetted to reintroduce the water of hydration of the clay and other minerals that, though not driven off at 105 degrees C, is lost at 500 degrees C. This water loss often amounts to ten percent of the weight lost during ignition and, if not corrected for, will be interpreted as organic matter. Subtract the weight of the crucible from the dry weight to obtain ash-free weight.

4.5.3 Gradient is the percent of slope of the stream bed which affects velocity and the ability of the stream to maintain substrate quality. Gradient is particularly important in streams and rivers where it influences siltation and scouring.

TABLE 2. SUBSTRATE PARTICLE SIZE CLASSIFICATION FOR SIEVE ANALYSIS*

Name	Particle Size (mm)	U.S. Standard Sieve Number
Boulder	>256	
Rubble	64 to 256	
Coarse Gravel	32 to 64	
Medium Gravel	8 to 32	Available but not U.S. Standard
Fine Gravel	2 to 8	10
Coarse Sand	0.5 to 2	35
Medium Sand	0.25 to 0.5	60
Fine Sand	0.125 to 0.25	120
Very Fine Sand	0.0625 to 0.125	230
Silt	0.0039 to 0.0625	See Text
Clay	<0.0039	See Text

*Modified from Wentworth, 1922; see Cummins, 1962.

4.5.4 Current velocity affects the distribution of organisms in lotic environments and along the windswept shores of lentic environments, both directly because of differing species requirements and indirectly by sorting of bottom sediments. Therefore, it is of critical importance that velocity be considered when sampling stations are selected, and when data are analyzed. Only stations with similar velocity should be compared. In addition, windswept and protected shores of lakes may not be comparable. At the actual time of sampling, velocity should be determined at each sampling point. Relatively inexpensive current meters are commercially available (See equipment list in Appendix E). Current velocity may also be determined by use of a home-made velocity head tube described by Ciborowski (1989).

4.5.4.1 At depths greater than three feet, use the two-point method; take readings at two points, 0.2 and 0.8 of the depth below the surface. The average of these two observations is taken as the velocity.

4.5.4.2 At depths less than three feet, take one reading at 0.6 of the depth. Where artificial substrate samplers or drift nets are being utilized, take the reading directly upstream of the sampler and at the same depth as the sampler.

4.5.5 Flow rate may be a factor in the distribution of benthic organisms in that it indirectly effects other factors such as current velocity and water depth. Also, flow rate is a factor in the dilution of toxic substances in the water. During periods of low flow a toxic material will cause greater stress on the organisms present because of higher concentration of the substance. For this reason it is often desirable to sample areas of suspected problems during low flow conditions in order to determine if an effluent is causing a stress on the aquatic community. Comparison of a sampling station during the sample period from year to year may not be valid if there is a large difference in flow rate between the two years.

4.5.6 Depth indirectly affects the distribution of aquatic macroinvertebrates as a result of its influence on the availability of light for plant growth, water temperature, the zonation of bottom deposits, water chemistry (particularly oxygen), and on phototactic responses of organisms. In selecting sampling stations for both qualitative and quantitative studies, depth must be measured and included as an independent variable in the study design.

4.5.7 Turbidity is defined as a cloudy condition in water due to the suspension of silt or finely divided organic matter. It is an important factor in that it directly effects light penetration and indirectly effects the productivity of algae and aquatic plants. The settling out of solids can also eliminate all life from a stream or river, or reduce its amount without greatly altering its composition simply by shading out all or some of the plant life, smothering out all algal growth, and altering the nature of the substratum.

4.5.8 Salinity is an important factor in marine and estuarine environments. The salinity of freshwater is generally a few parts per million compared to approximately 35 parts per thousand for sea water. Where sea water and fresh water meet in estuaries, there may be wide fluctuations of salinity due to variations in tides and river discharge, and a salt wedge may extend upstream under the fresh water layer for a significant distance. This area may be inhabited to some extent by both freshwater and saltwater forms, but the number of species is usually less than that under more stable conditions of salinity (Macan, 1963). Since movement and location of many species is governed by tides and salinity, these must be taken into account in determining sampling location as well as time of sampling.

4.5.8.1 Because of the extreme spatial and temporal fluctuations of salinity in the estuaries, simple, rapid instrumental measurements are more desirable than slower, more precise chemical methods (Mangelsdorf, 1967). Wide range, temperature-compensated conductivity salinometers are recommended for determining both horizontal and vertical salinity profiles at high-slack and low-slack tide levels in the area of estuary or reach of river being studied.

4.5.9 Tidal inundation (the amount of time that a particular stratum is inundated in marine intertidal zones) affects the kinds of organisms that can live within the substrate. Organisms that can resist desiccation and temperature change are able to colonize the intertidal zone. Organisms that cannot, will be restricted to the sublittoral zone or area below the tidal reach.

4.5.10 Chemical factors such as alkalinity, pH, hardness and sulfates are also important factors to consider. They affect the numbers and composition of macroinvertebrates in the stream. Alkalinity is closely related to primary productivity. An increase in sulfates causes deterioration in water quality and adversely affects the macroinvertebrate community.

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SECTION 5

SAMPLING METHODS

5.1 Introduction

5.1.1 Aquatic macroinvertebrates are good indicators of environmental water quality in fresh, estuarine, and marine waters. The analysis of faunal assemblages is an excellent way to detect water quality problems. Different kinds of stress will often produce different communities of benthic macroinvertebrates. The sampling equipment and methods discussed can be used to study and analyze macroinvertebrate communities for ambient or special studies, and the resulting data and information can be used to document both spatial and temporal changes in water quality. The sampling devices and methods of this section relate to qualitative, semi-quantitative, and quantitative sampling.

5.1.1.1 Qualitative and semi-quantitative sampling of macroinvertebrates are relatively easy. The current methodology discussed here is well developed, and the equipment needed for sampling is not elaborate. Many effective methods of data analysis, including pollution indices and diversity indices, have been developed for use with macroinvertebrates (also, see Section 7, Data Evaluation).

5.1.1.2 Quantitative sampling is more difficult. Random sampling and the patchy distribution of macroinvertebrates within the substrate often means larger numbers of samples are needed in order to be able to make reasonable estimates of community structure and population densities. However, this is not a problem confined only to macroinvertebrates, but to other aquatic animals as well. Also, see Section 4, Selection of Sampling Sites and Section 7, Data Evaluation.

5.1.2 The sampling methods employed should depend on the data quality objectives (DQOs) (see Section 2, Quality Assurance and Quality Control) of the study determined by interaction of the decision making authority and biomonitoring expertise of qualified aquatic biologists.

5.1.3 A list of equipment, supplies, and companies that can provide sampling gear for collecting benthic macroinvertebrates can be found in Appendix E.

5.2 Qualitative Sampling

5.2.1 The objective of qualitative studies is to make within or between site comparisons to determine the presence or absence of benthic macroinvertebrates having varying degrees of tolerance to pollution and to obtain information on the richness of taxa, at or near the species level (taxa present and relative abundance). Samples are obtained with the use of a wide variety of collecting methods and gear, many of which are not amenable to quantification on a unit-area or volume basis. Any collecting device (e.g., dip or hand nets, kick nets, screens, dredges, grab samplers, stream-net samplers, and artificial substrate samplers) can be used for qualitative collections of macroinvertebrates. The use of several methods of collection at each station can increase the total number of taxa collected. When conducting qualitative studies, an attempt is usually made to collect as many taxa as possible in the time available by exhaustive sampling in all available habitat types. No habitat should be overlooked at the site if the objective of the study is to obtain a representative collection of the macroinvertebrates.

5.2.2 Experience and skill are required in selecting suitable collecting techniques and recognizing and locating various types of habitats where qualitative samples can be collected.

5.2.3 When conducting comparative studies of the macrobenthos, a major drawback is the confounding effect of the differences in physical habitat among the different stations being studied. This problem is particularly inherent in qualitative studies when an attempt is made to systematically collect as many species as possible at the sampling stations or reaches of streams being compared. Unfortunately, differences in habitat unrelated to the effects of pollution may render such comparisons meaningless. To minimize this drawback, the investigator should carefully record, in the field, the habitats from which specimens are collected (a habitat assessment) and then base comparisons only on stations with like habitats in which the same amount of collecting effort has been expended. Appropriate sampling methods, such as the use of artificial substrates, should be utilized to eliminate the problem of comparing different physical habitats among stations being studied.

5.2.4 Advantages of qualitative sampling are the wide latitude in collecting methods, the types of habitats that can be sampled are relatively unrestricted, and the processing of the samples is usually less time consuming.

5.2.5 Limitations of qualitative sampling include collecting techniques that are subjective and depend on the skill and experience of the sample collector, sampling results of one investigator can be difficult to compare with those of another, and no information on standing crop or biomass can be generated from a qualitative study.

5.3 Semi-quantitative Sampling

5.3.1 Semi-quantitative sampling data can be generated based on methods that measure the collection of benthic macroinvertebrates by level of effort (e.g., time expended per habitat) or when quantitative sampling devices are used to collect samples in a non-random manner. Examples of some semi-quantitative methods include the 10 rock method (Lewis, personal communication), traveling kick method (Hornig and Pollard, 1978; Pollard, 1981), and Rapid Bioassessment Protocols II and III (Plafkin *et al*., 1989). See Section 7, Data Evaluation.

5.4 Quantitative Sampling

5.4.1 Quantitative methods essentially provide an estimation of the numbers or biomass (standing crop) of the various components of the macroinvertebrate community per unit area, volume, or sampling unit. The method also provides information on the species composition, richness of species, and distribution of individuals among the species. The high variability often associated with some macroinvertebrate populations makes them difficult to study quantitatively (Schwenneker and Hellenthal, 1984), but multi-metric assessment endpoints are used to avoid the difficulty of utilizing only population-based measurement endpoints. Section 7, Data Evaluation and Elliott (1971) should provide statistical principles for sampling and data analyses of benthic macroinvertebrates.

5.4.2 Quantitative estimates are obtained by using devices that sample a unit area or volume of the habitat. The major considerations are the size of the sampling units, the number of sampling units in each sample, and the location of sampling units in the sampling area. Grab samplers, stream-net samplers (e.g., Surber and related type samplers, Hess and related type samplers, and drift nets), and artificial substrate type samplers, are examples of devices that are used to collect samples quantitatively.

5.4.3 Sampling precision in the study of macroinvertebrate populations is affected by the substrate area encompassed by the sampling device and the patchiness in distribution of the organisms. The smaller the substrate surface area encompassed by a sampling device, the larger the number of sampling units required to obtain a desired level of precision (Elliott, 1971). Precision can be increased by collecting larger sampling units or by increasing the numbers of sampling units collected. A quantitative approach necessitates that a measure of the precision be obtained by replicate sampling. Replicate sampling in each habitat (habitat niche, microhabitat, or strata) selected for study is an absolute requirement.

5.4.3.1 For measurement of precision, three replicate random sampling units at each sampling station are an absolute minimum. Five replicates at each station would increase the statistical precision and accuracy. A series of single sampling units taken at discrete points along a transect do not represent replicate samples of benthic organisms unless it can be demonstrated that the physical characteristics of the habitat do not change along the transect.

5.4.4 The total number of samples depends on the degree of precision required, which will depend on the type of study and data quality objectives (DQOs). A reconnaissance or pilot study of the station may be necessary to help determine the sample size. Southwood (1966) gives a formula for determining the number of sampling units required for a specific level of precision.

5.4.5 The data from properly designed quantitative studies are amenable to the use of simple but powerful statistical tools that aid in maintaining the objectivity of the data evaluation process (see Section

7, Data Evaluation). The measures of precision and probability statements that can be attached to quantitative data reduce the possibilities of bias in the data evaluation process and make the results of different investigators more readily comparable. The advantages of quantitative methods are that they provide a measure of invertebrate diversity, biomass, and productivity, and their associated precision, thereby providing objective comparisons within, between, and among studies or intra- and interstudy comparisons.

5.4.6 No one sampling device is completely adequate to sample all types of habitat. When either qualitative, semi-quantitative, or quantitative devices are used, only selected portions of the environment are usually sampled. Also, because of the potential use of these data, experienced and skilled biologists are needed for sample collections.

5.5 Sampling Devices

5.5.1 Grab Samplers (Grabs)

5.5.2 Grabs are devices designed to penetrate the substrate by virtue of their own weight and leverage and have spring- or gravity-activated closing mechanisms. The jaws of grabs are forced shut by weights, lever arms, springs, or cables. All grabs are designed to take discrete "bites" or "scoops" of a defined area into the bottom sediment of a lake, stream, estuary, ocean, or similar habitats to sample the benthos. Scoops are grab samplers that scoop sediment with a rotating container. In shallow waters, some of these devices may be rigged on poles or rods and physically pushed into the substrate to a predetermined depth.

5.5.2.1 The number and kinds of macroinvertebrates collected by a particular grab may be affected by the habitat sampled, substrate type sampled, depth of penetration, angle of closure, completeness of closure of the jaws and loss of sample material during retrieval, creation of a "shock" wave and consequent "washout" of organisms at the surface of the substrate, and the effect of the high-flow velocities often encountered in rivers and wave action in large lakes and oceans on the stability of the sampler.

5.5.2 Selecting Grab Sampling Devices

5.5.2.1 Table 3 summarizes criteria for selecting grabs.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS

1. Ponar Grab (Standard)

   A. Habitats and Substrates Sampled: Freshwater lakes, rivers, estuaries, and reservoirs with hard and soft sediments such as clay, hard pan, sand, gravel and muck; somewhat less efficient in softer sediments.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS (Continued)

B. Effectiveness of the Device: Not entirely adequate for deep burrowing organisms in soft sediments; very efficient for hard sediments; collects both qualitative and quantitative samples.

C. Advantages: Better penetration than other grabs; side plates and screens reduce washout, shock waves and substrate disturbance; best quantitative grab sampler for freshwater use.

D. Limitations: A very heavy grab that requires use of a boat with winch and cable; stones, pebbles and other debris can hold jaws open causing loss of sample.

2. Petite Ponar Grab

A. Habitats and Substrates Sampled: Freshwater lakes, rivers and reservoirs and estuaries with moderately hard sediments such as sand, silt and mud; will not penetrate clay; somewhat less efficient in soft sediments and coarse gravel.

B. Effectiveness of the Device: Not entirely adequate for deep burrowing organisms in soft sediments; not useful in clay.

C. Advantages: Good penetration for such a small grab; side plates and screens reduce washout, shock waves and substrate disturbance; can be operated by hand without boat or winch.

D. Limitations: Jaws can be blocked by stones, sticks and other debris causing loss of part of the sample; not efficient in swiftly flowing water of over one meter per second velocity.

Selected Literature: APHA, 1989; ASTM, 1990; Brinkhurst, 1967, 1974; Elliott _et al_., 1978, 1980, 1981b; Flannagan, 1970; Howmiller, 1971; Hudson, 1970; Lewis _et al_., 1982; Powers and Robertson, 1967; USEPA, 1973.

3. Ekman Grab (Standard, Tall, Large, and Extra-large)

A. Habitats and Substrates Sampled: Freshwater rivers, lakes and reservoirs where there is little current; soft sediments such as muck and silt.

B. Effectiveness of the Device: Efficient only in soft sediments but weights can be added for deeper penetration in fine sand; collects both qualitative and quantitative samples.

C. Advantages: Easy to operate by hand without winch, can be pushed into substrate in shallow water; hinged doors at top reduce washout, shock waves and disturbance of the substrate; comes in a range of sizes.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS (Continued)

D. Limitations: Light weight so that jaw will not penetrate hard substrates; jaws often do not close completely due to blocking of jaws or failure of closing mechanism; inefficient in deep water or where there is even moderate current.

Wildco box corer resembles a heavy duty Ekman grab that has been designed to penetrate harder substrates with the addition of a frame and weights. The device can be used to collect infauna of lakes and estuaries. The box corer may also be used to sample finely divided muck, clays, mud, ooze, submerged marl, or fine peaty bottoms. The sampler weighs about 14 kg, but a maximum of 49 kg (12 removable weights) may be used. The sample area is 150 x 150 x 225 mm; a removable acrylic liner is included.

Selected Literature: APHA, 1989; ASTM, 1990; Beattie, 1979; Burton and Flannagan, 1973; Ekman, 1911, 1947; Flannagan, 1970; Howmiller, 1971; Hudson, 1970; Lanz, 1931; Lewis _et al_., 1982; Lind, 1974; Milbrink and Wiederholm, 1973; Paterson and Fernando, 1971; Rowe and Clifford, 1973; Rawson, 1947; Schwoerbel, 1970; Welch, 1948; USEPA. 1973.

4. Petersen Grab (Standard and Baby)

A. Habitats and Substrates Sampled: Freshwater lakes, reservoirs and rivers and estuaries with sand, gravel, clay and hard pan substrates.

B. Effectiveness of the Device: Less effective in most substrates than the Ponar, Baby Petersen effective in moderately soft sediments.

C. Advantages: Can give quantitative samples if used properly; range of sizes available.

D. Limitations: Standard grab is heavy and requires boat with winch; can cause washout if dropped rapidly to the bottom; shallow bite by jaws so that deeper burrowing organisms are not sampled; jaws are easily blocked by debris causing loss of sample; hard to use in adverse weather; of questionable value as a quantitative sampler.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Birkett, 1958; Brinkhurst, 1974; Davis, 1925; Edmondson and Winberg, 1971; Elliott and Tullett, 1978; Holme and McIntyre, 1971; Hudson, 1970; Howmiller, 1971; Jensen, 1981; Lewis et al., 1982; Petersen, 1918; Petersen and Tensen, 1911.

5. Smith-McIntyre Grab

A. Habitats and Substrates Sampled: Marine and estuaries; adaptable to large rivers, lakes and reservoirs with sand, gravel, clay and similar substrates.

B. Effectiveness of the Device: Limited penetration; has been widely used for sampling in marine and estuarine habitats.

C. Advantages: Provides reasonably quantitative samples; trigger plates help penetrate the substrate.

D. Limitations: Very heavy, needs boat and power winch; spring loaded jaws could be hazardous; inefficient for collecting deep burrowing organisms; jaws can be blocked by debris.

Selected Literature: APHA, 1989; ASTM, 1990; Carey and Heyamoto, 1972; Carey and Paul, 1968; Elliott and Tullett, 1978; Holme, 1964; Hopkins, 1964; Hunter and Simpson, 1976; McIntyre, 1971; Smith and McIntyre, 1954; Tyler and Shackley, 1978; Wigley, 1967; Word, 1976, 1977; Word et al., 1976.

6. Van Veen Grab

A. Habitats and Substrates Sampled: Marine and estuaries with sand, gravel, mud, clay and similar substrates; could be adapted to freshwater.

B. Effectiveness of the Device: Penetrates to a depth of 5 to 7 cm.

C. Advantages: Jaws close better than the Petersen Grab; samples most types of sediments; comes in a range of sizes.

D. Limitations: A very heavy grab that requires a large boat and power winch; jaws may become blocked by debris such as rocks and sticks; not useful for deep burrowing organisms.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Beukema, 1974; Birkett, 1958; Elliott and Drake, 1981b; Elliott and Tullett, 1978; Holme, 1971; Lassig, 1965; Longhurst, 1959; McIntyre, 1956; Nichols and Ellison, 1966; Schwoerbel, 1970; Ursin, 1954; Wigley, 1967; Word, 1976a, 1976b; Word et al., 1976.

7. Orange-Peel Grab

A. Habitats and Substrates Sampled: Marine waters and deep lakes with sandy substrates containing cobble, rubble and coarse gravel.

B. Effectiveness of the Device: For qualitative use only; sampling area not constant.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS (Continued)

C. Advantages: Comes in a range of sizes; works well in deep water; closes relatively well to prevent loss of sample; good for reconnaissance.

D. Limitations: Very heavy so that large boat with power winch and cable lines is required; does not sample constant area and depth.

Selected Literature: APHA, 1989; ASTM, 1990; Briba and Reys, 1966; Elliott and Tullett, 1978; Hartman, 1955; Hopkins, 1964; Merna, 1962; Packard, 1918; Reish, 1959; Thorson, 1957, Word, 1976, 1977.

8. Shipek Grab

A. Habitats and Substrates Sampled: Estuaries and large deep lakes with sand, gravel, mud and clay substrates.

B. Effectiveness of the Device: A relatively good quantitative sampler.

C. Advantages: Good for collecting a small sample in deep water.

D. Limitations: A heavy grab that requires the use of a boat with a power winch; must be lowed vertically so is not effective in moving water; inefficient for collecting deep burrowing organisms; samples small area.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Elliott and Tullett, 1978; Flannagan, 1970; Holme, 1964, 1971; Holme and McIntyre, 1971.

5.5.3 Precautions

5.5.3.1 Always inspect grabs for mechanical defects prior to use.

5.5.3.2 Exercise caution at all times when handling grabs.

5.5.4 Significance and Use of Grabs

5.5.4.1 Qualitative and quantitative samples of macroinvertebrates inhabiting sediments or substrates are may be taken by grabs. Grab samplers, if used correctly, are devices that sample a unit area of the habitat. In view of the advantages and limitations regarding the penetration of the sediment by many grabs and their closing mechanisms, it is not possible to recommend any single instrument as suitable for general use. However, the Petersen grab is considered the least effective bottom grab sampler and, therefore, has limited application. The type and size of the grab sampler or device selected for use will depend on such factors as the size of boat, hoisting gear available, the type of substrate or sediment to be sampled, depth of water, current velocity, and whether sampling is in sheltered areas or in open waters of large rivers, reservoirs, lakes, or oceans. The choice of grab will depend largely on what is available, what is suitable for the sampling area, and what can be used with the least difficulty.

5.6 Commonly Used Grabs

5.6.1 The ponar grab sampler (Fig. 5A,B) is most commonly used for sampling macroinvertebrates from sediments in lakes, rivers, reservoirs, estuaries, and oceans with coarse and hard substrates, such as coarse sand, gravel, and similar substrates, rather than soft sediments, such as mud, fine sand, or sludge. The sampler can be used in moderate currents and deep waters.

5.6.1.1 The Ponar grab sampler has paired jaws that must penetrate beneath the surface of the substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The device has side plates and a screen on the top of the sample compartment to prevent loss of the sample during closure. With one set of weights, this heavy steel sampler can weigh 20 Kg. Word _et al_. (1976a) reports that the large amount of surface disturbance associated with Ponar grabs can be greatly reduced by simply installing hinges rather than fixed screen tops, which will reduce the pressure wave associated with the sampler's descent into the sediment. The standard Ponar takes a sample area of 523 cm^2. A small version, the petite Ponar grab, takes a sample area of 232 cm^2 and can be used in habitats where there may be an unusual abundance of macroinvertebrates, thus eliminating the need to subsample.

5.6.1.2 When not in use, a safety pin lock attached to the lever bar prevents closing of the sampler until the pin is removed.

5.6.1.3 The weight of the standard Ponar grab makes it necessary to use a winch and cable or portable crane for retrieving the sample, and ideally the samples should be taken from a stationary boat or platform. The smaller version, petite Ponar grab, is designed for hand-line operation, but it may be used with a winch and cable.

5.6.2 The Ekman grab sampler (Fig. 5C) is used to obtain samples of macroinvertebrates from soft sediments, such as very fine sand, mud, silt, and sludge, in lakes, reservoirs, estuaries, and similar habitats where there is little current. This grab is inefficient in deep waters, under adverse weather conditions, and in waters of moderate to strong currents or wave action. The Wildco box corer (Fig. 5D) is like a heavy duty Ekman with a frame and weights and is used to collect macroinvertebrates in lakes and estuaries. Because of its weight a winch is necessary for retrieving the sample from a stationary boat or platform.

5.6.2.1 The Ekman grab sampler is a box-shaped device with two scoop-like jaws that must penetrate the intended substrate without disturbing

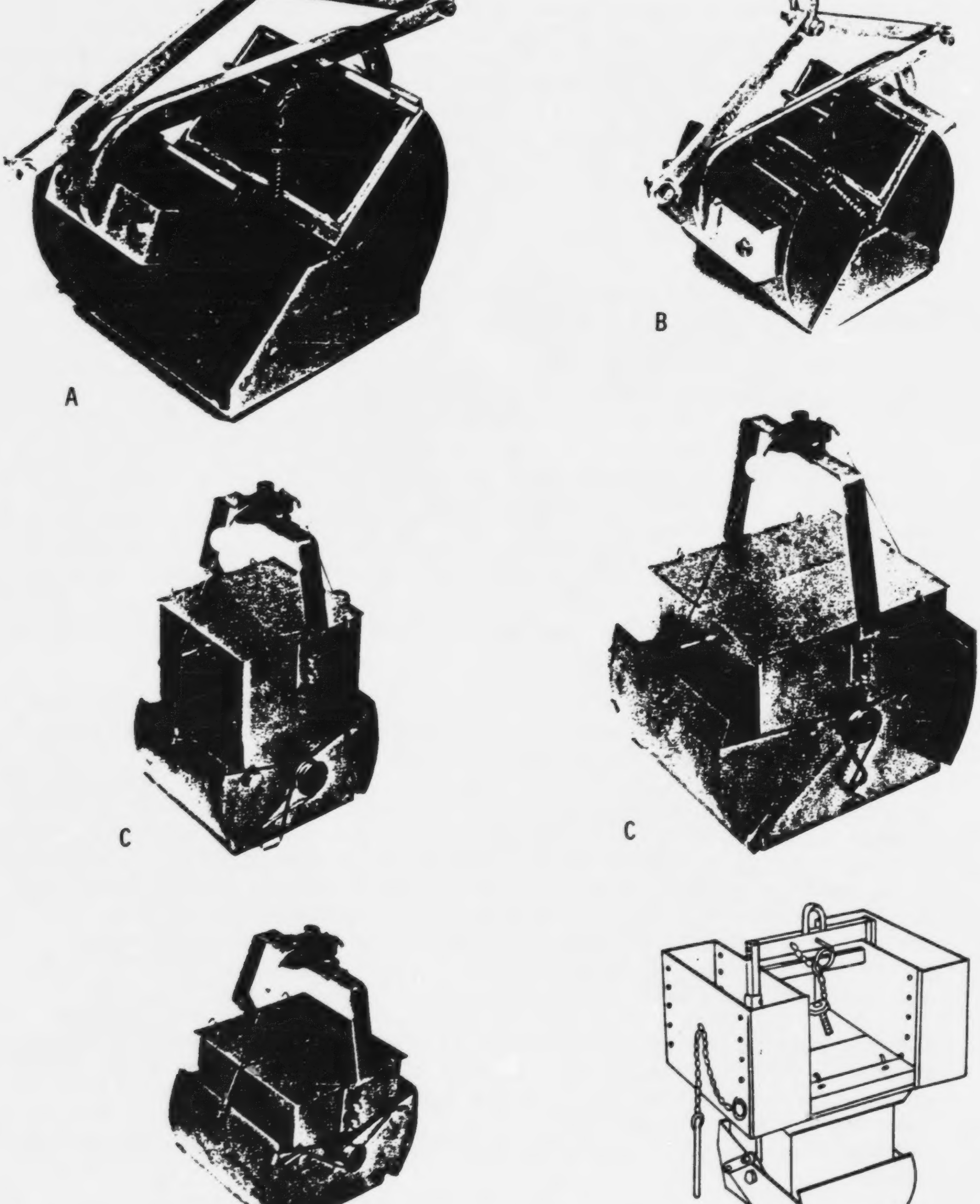

Figure 5. Grab Samplers. (A) Standard Ponar; (B) Petite Ponar; (C) Large, tall, and standard Ekman grabs; (D) Wildco box corer

the water surface boundary layer, close when positioned properly on the bottom, and retain a discrete sample of sediment while it is brought to the surface for processing. Hinged doors on the top of the grab prevents washout during sample lowering and retrieval. The grab is made of 12 to 20 gauge brass or stainless steel and weighs approximately 3.2 Kg. The box-like part holding the sample has spring-operated jaws on the bottom that must be manually set. The sampler is available in several sizes; however, in very soft substrates only a tall model should be used, either a 23 cm or a 30.5 cm model. Ekman is not used with a winch very often but can be operated from a boat with a winch and cable.

5.6.2.2 Exercise caution at all times once the grab is loaded or cocked because a safety lock is not part of the standard design.

5.6.3 The Petersen grab sampler (Fig. 6A,B) is designed to obtain samples of macroinvertebrates from sediments in lakes, reservoirs, and similar habitats and is adaptable to rivers, estuaries, and oceans. This grab sampler has limited application, and is not recommended for quantitative benthic work and must be used with due consideration of its defects when quantitative estimates are attempted. It is useful for sampling sand, gravel, marl, and clay in moderate currents and deep waters, the sampler cannot be used under adverse weather conditions. This sampler is available in a range of sizes that will sample an area from 0.06 to 0.099 m^2. A consensus of aquatic biologists consider the use of this device the least preferable grab sampler and would use it only in limited applications.

5.6.3.1 The Petersen grab sampler has paired jaws that must penetrate the intended substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain the sample of sediment while it is brought to the surface for processing. This heavy steel device can weigh 13.7 Kg, but may weigh as much as 31.8 Kg when auxiliary weights are bolted to its side. The extra weights are to make the grab stable in swift current and to give additional cutting force in firm bottom sediments. It has been suggested that users of this device modify it by the addition of end plates and by cutting large strips out at the top of each side and adding hinged 30 mesh screen as in the Ponar grab. It is necessary to use a winch and cable to lower and raise the sampler.

5.6.3.2 Newer versions of the Petersen grab sampler may have a screened window at the top of each jaw to allow water to escape while the grab is descending and closing. While some modifications may close or function better, the sampling characteristics remain the same. Most of the modified versions are intended for use in estuarine and marine waters.

5.6.3.3 Ideally a stationary boat or platform should be used when taking samples. The modified Petersen devices are designed to be quite heavy and require heavy gear and a large vessel for efficient operation. A small version can be hauled aboard by hand and held with one hand for washing procedures.

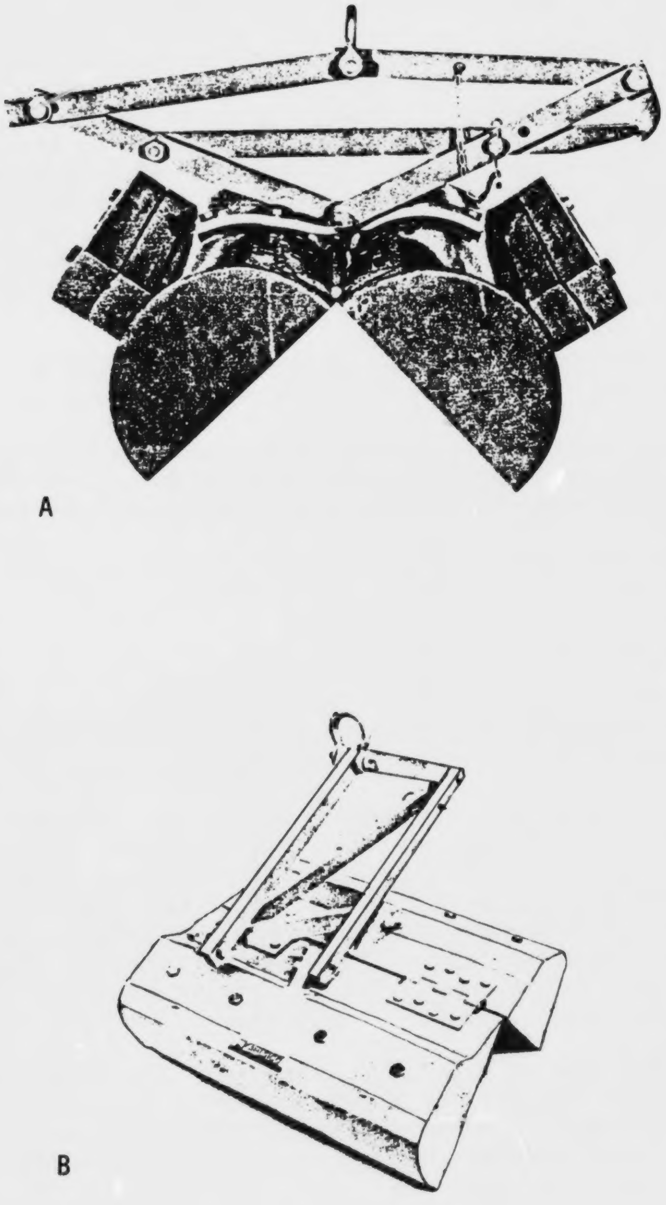

Figure 6. Grab Samplers: (A) Original Petersen; (B) Modified Petersen

5.6.4 The Smith-McIntyre grab sampler (Fig. 7A) is designed to obtain samples of macroinvertebrates from sediments in rough weather and deep water in lakes, rivers, estuaries, and oceans. This device samples a surface area of 0.1 m^2 and is useful for sampling macroinvertebrates from sand, gravel, mud, clay, and similar substrates.

5.6.4.1 The Smith-McIntyre grab sampler has paired jaws that are forced to penetrate into the intended substrate by two "loaded" strong coiled springs, must close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The device is heavy and can weigh 45.4 Kg or more. The chief advantages of the sampler are its stability and easier control in deep and rough waters. The spring-loaded jaws of the Smith-McIntyre grab must be considered a hazard and caution should be exercised when using the device. Due to the weight and size, this device must be used from a vessel with boom and lifting capabilities.

5.6.4.2 The Smith-McIntyre grab sampler is fitted with gauze panels or free swinging panels on the top to reduce the shock wave during descent.

5.6.4.3 Larger Smith-McIntyre grabs can be constructed depending on the type of bottom to be sampled and additional weights can be fitted to the frame of the grab sampler for additional penetration into the sediment.

5.6.5 The Van Veen grab sampler (Fig. 7B) is used to obtain samples of macroinvertebrates from sediments in estuaries and other marine habitats, and is adaptable to freshwater areas. It can also be used for qualitative sampling. This device is useful for sampling sand, gravel, mud, clay and similar substrates and is available in two sizes, 0.1 m^2 and 0.2 m^2. Larger and double versions of this grab are available, and their use is dependent upon the type of bottom to be sampled, and the type of vessel available to deploy this sampler.

5.6.5.1 The Van Veen grab sampler has paired jaws that must penetrate the intended substrate without disturbing the water surface boundary layer of the substrate, close by pincher-like action of two long arms when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The long arms give added leverage for penetrating hard sediments. The advantage of using the twin Van Veen is that with a single lowering, two separate bottom sediment sampling units can be collected from the same station.

5.6.5.2 The Van Veen is basically an improved version of the Petersen grab in that long arms have been attached to the jaws to stabilize the grab on the bottom in the open sea just prior to or during closure of the device. Additional weights can be applied to the jaws to effect greater penetration in sediments.

5.6.6 The Orange-Peel grab sampler (Fig. 7C) is used primarily in marine waters and deep lakes where it has advantages over other grabs when sandy substrates are sampled, but it cannot be used under adverse weather conditions. This grab should not be used in critical quantitative work that is to be compared with results of other areas and is recommended as a reconnaissance sampler only. The sampler is available in a range of sizes but the 1600 cm^3 is generally used, although larger sizes are available.

5.6.6.1 The Orange-Peel grab sampler has four curved jaws that close to encircle a hemisphere of sediment. It must penetrate the intended substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The top of the sampler is enclosed by a canvas bag, serving as a portion of the sample compartment. When taking samples, a stationary boat or platform should be used.

5.6.6.2 A recent modification of the Orange-Peel, described by Reish (1959) has a new trigger mechanism and more efficient closing jaws, and the volume of sample to surface-area sampled relationship has been worked out.

5.6.6.3 The surface area sampled by this device varies with penetration depth or volume sampled. The device penetrates to a maximum depth of 18 cm, but depth of penetration will vary.

5.6.7 The Shipek (scoop) grab sampler (Fig. 7D) is designed to obtain samples of macroinvertebrates from sediments in marine waters and large inland bodies of water. This device is useful for sampling macroinvertebrates from sand, gravel, mud, clay, and similar substrates. It is designed to take a sediment sample with a surface area of 20 cm^2 to approximately 10 cm deep at the center.

5.6.7.1 The Shipek (scoop) grab sampler consists of a semi-cylindrical scoop that must be positioned properly on the bottom to take a scoop and retain discrete samples of sediment through 180°. Holmes and McIntyre (1971) report that this device is usually used by geologists to collect small samples rather than by biologists. However, it can be used in marine waters and large inland lakes, reservoirs, and rivers. Unlike many other types of samplers, closure of the device is made at the side, rather than at the bottom. This sampler cannot be used under adverse wind and wave conditions. The sampler requires a vessel with a winch and cable.

5.6.8 General Operating Procedures

5.6.8.1 Most grabs are heavy sampling devices that should be operated using a hand or powered winch and cable from a boat. In large bodies of water ships are used for this operation.

5.6.8.2 Grabs must be lowered slowly because free-fall may airplane the device, causing the device to land improperly or causing a pressure wave and blowout of the surface layer of sediment when the grab reaches the

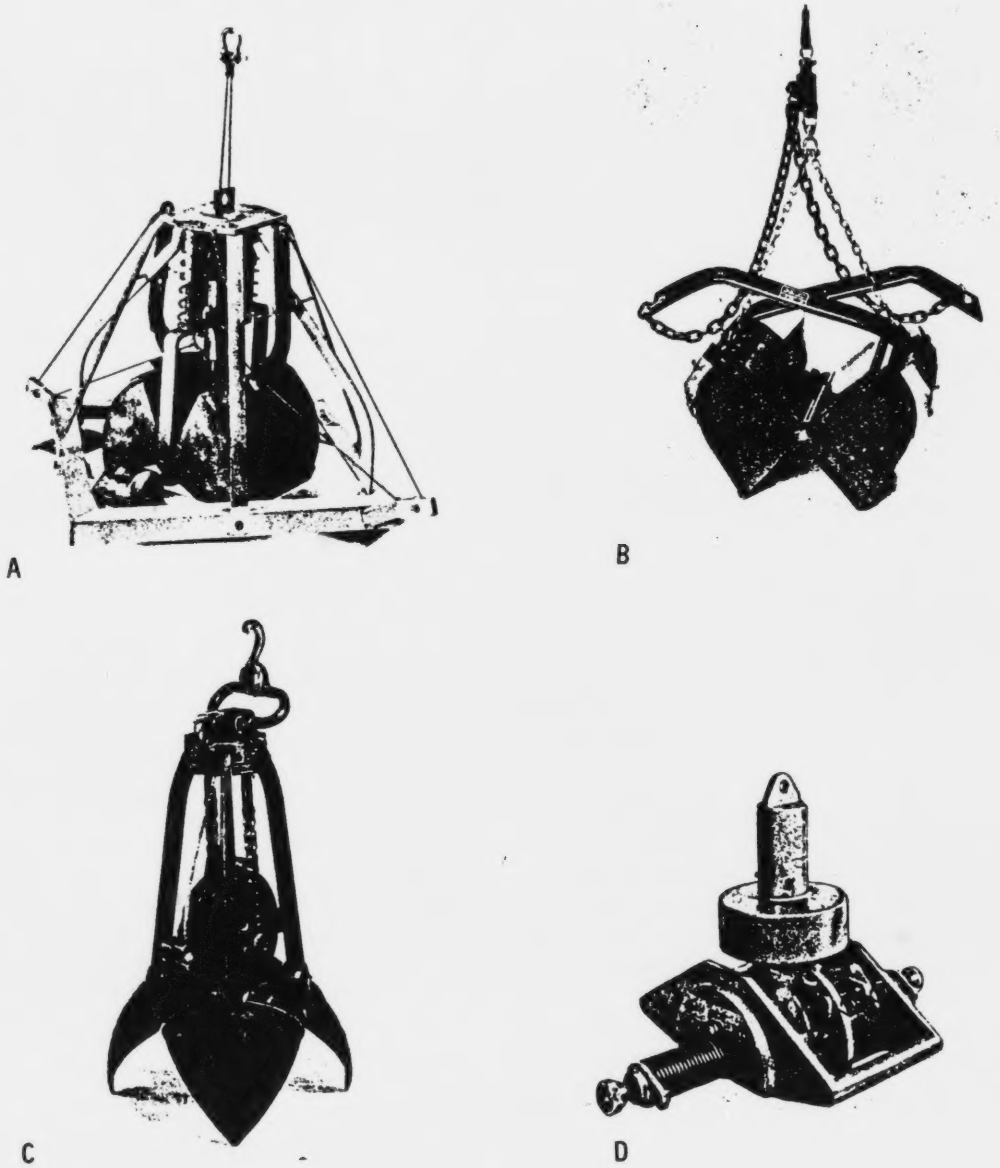

Figure 7. Grab Samplers: (A) Smith-McIntyre; (B) Van Veen; (C) Orange-Peel; (D) Shipek

bottom. In order for the device to operate effectively, it must bite vertically.

5.6.8.3 When most grabs reach the bottom, their weight will cause them to penetrate the substrate, and the slack-off on the cable allows the locking lever to release, therefore permitting the movement that allows the horizontal locking bar to drop out of the locking notch and allow the jaws to close as the device is raised. Other grabs are closed by spring action or some other mechanical device after penetrating the substrate.

5.6.8.4 In the Ekman grab the jaws are cocked by raising them upward into the cocked position using the attached cable and securing the cable to the catch pin located at the top of the sampler. Once on the bottom, indicated by a slack line, a messenger is sent down the line tripping the catch mechanism, causing the spring loaded jaws to close the bottom of the sampler and contain the sediment.

5.6.8.5 The Smith-McIntyre grab is "loaded" by compressing the large coil springs mounted on the instrument with the loading bar. As soon as the spring is loaded, the safety pin is inserted to prevent the accidental triggering of the bottom plates. Once the device is overboard, just prior to lowering to the bottom, the safety pins are removed. When the trigger plates contact the bottom, pressure on these plates releases the two coiled springs that drive the buckets (jaws) into the sediment. Closure of the sampler is made at the side, rather than at the bottom. After closure the sample is given optimum protection from washout during the return trip to the surface by the cylindrical configuration of the sampler. Once on deck, the sampler is placed on a stand; the sample buckets can be disengaged from the rest of the device by releasing two retaining latches at each end of the upper semicylinder, and the sample is dumped into a large basin or washtub and prepared for processing. After the sample has been removed, the springs may then be loaded and the safety pins installed.

5.6.8.6 The chains from the jaws of the Van Veen are attached to the counter balance mechanism, as are the slackened wires from the long arms. Tension is carefully applied to the trigger mechanisms as the sampler is winched off its platform, and once the tension is firmly changed from the jaws, the grab is relatively stable in the cocked position. Care should be exercised in lowering the Van Veen through the surface of the water as occasionally contact will produce slack in the chain that will trip the counter balance mechanism. The grab is lowered slowly to the bottom, and once it makes contact with the bottom, the grab is winched in initially closing the jaws containing the sediment. Retrieve the grab slowly to prevent washout.

5.6.8.7 The Shipek grab is composed of two concentric half cylinders, the inner semicylinder is rotated at high torque by two spirally wound external springs. Upon contact with the bottom, the two external springs are automatically released by the inertia of a self-contained weight upon a sear mechanism which trips the catch and the scoop rotates upward. At the end of its 180° travel, the sample bucket is stopped and held at the closed position by residual spring torque. After closure the sample is given optimum protection from washout. The scoop is disengaged from the upper semicylinder by releasing the two retaining latches at each end of the upper semicylinder.

5.6.8.8 Once on board, the sample is placed into either a suitable container or a sieving device directly for processing (see Section 6). Thoroughly wash or hose the grab with water, so that all sediment materials are included in the sample before a replicate sample is taken.

5.7 Stream-Net Samplers

5.7.1 Stream-net samplers are lotic collecting devices, fitted with a net of various mesh sizes that collect organisms from flowing water passing through the sampler.

5.7.2 Selecting Stream-Net Sampling Devices

5.7.2.1 Table 4 summarizes criteria for selecting stream-net sampling devices.

TABLE 4. SUMMARY CRITERIA FOR STREAM-NET SAMPLERS

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1. Surber Sampler

   A. Habitats and Substrates Sampled: Shallow, flowing streams, less than 32 cm in depth with good current; rubble substrate, mud, sand, gravel.

   B. Effectiveness of Device: Relatively quantitative when used by experienced biologist; performance depends on current and substrate.

   C. Advantages: Encloses area sampled; easily transported or constructed; samples a unit area.

   D. Limitations: Difficult to set in some substrate types, that is, large rubble; cannot be used efficiently in still, slow moving streams.

2. Portable Invertebrate Box Sampler, Hess Sampler, Hess Stream Bottom Sampler, and Stream-Bed Fauna Sampler

   A. Habitats and Substrates Sampled: Same as Surber.

   B. Effectiveness of Device: Same as Surber.

   C. Advantages: Same as above except completely enclosed with stable platform; can be used in weed beds.

TABLE 4. SUMMARY CRITERIA FOR STREAM-NET SAMPLERS (continued)

D. Limitations: Same as Surber.

Selected Literature: APHA, 1989; ASTM, 1990; Canton and Chadwick, 1984; Elliott and Tullett, 1978; Ellis and Rutter, 1973; Hess, 1941; Kroger, 1972; Lane, 1974; Merritt _et_ _al_., 1984; Needham and Usinger, 1956; Pollard and Kinney, 1979; Rutter and Ellis, 1977; Rutter and Poe, 1978; Rutter and Ettinger, 1977; Resh, 1979; Resh _et_ _al_., 1984; Schwenneker and Hellenthal, 1984; Surber, 1937, 1970; Usinger, 1963; Waters and Knapp, 1961; Welch, 1948; Winner _et_ _al_., 1980.

3. Drift Nets

A. Habitats and Substrates Sampled: Flowing rivers and streams; all substrate types.

B. Effectiveness: Relatively quantitative and effective in collecting all taxa which drift in the water column; performance depends on current velocity and sampling period.

C. Advantages: Low sampling error; less time, money, effort; collects macroinvertebrates from all substrates, usually collects more taxa.

D. Limitations: Unknown where organisms come from; terrestrial species may make up a large part of sample in summer and periods of wind and rain; does not collect non-drifting organisms.

Selected Literature: Allan, 1984; Allan and Russek, 1985; APHA, 1989, ASTM, 1990; Bailey, 1964; Berner, 1951; Brittain and Eikeland, 1988; Chaston, 1969; Clifford, 1972a,b; Coutant, 1964; Cushing, 1963, 1964; Dimond, 1967; Edington, 1965; Elliott, 1965, 1967; 1969, 1970; 1971; Elliott and Minshall, 1968; Ferrington, 1984; Hales and Gaufin, 1969; Hemsen, 1956; Hildebrand, 1974; Holt and Waters, 1967; Hynes, 1970; Keefer and Maughan, 1985; Larimore, 1972, 1974; Larkin and McKone, 1985; Lehmkuhl and Anderson, 1972; McLay, 1970; Merritt _et_ _al_., 1984; Minshall and Winger, 1968, Modde and Schulmbach, 1973, Muller, 1965, 1974, Mullican _et_ _al_., 1967; Mundie, 1959, 1964; Pearson and Franklin, 1968; Pearson and Kramer, 1969, 1972; Pearson et al., 1968; Pfitzer, 1954; Radford and Hartland-Rowe, 1971; Reisen and Prins, 1972; Resh, 1979; Resh _et_ _al_., 1984; Spence and Hynes, 1971; Tanaka, 1960; Tranter and Smith 1968; USEPA, 1973; Waters, 1961, 1962, 1964, 1965; 1966; 1968, 1969a,b, 1972; Wilson and Bright, 1973; Winner _et_ _al_., 1980; Wojtalik and Waters, 1970.

5.7.3 The Surber, portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers (Fig. 8A-E) were designed as quantitative samplers when carefully used by an experienced biologist; however, they are more often used to collect qualitative samples or semi-quantitative samples because of the large number of samples needed for an acceptable level of precision (Needham and Usinger, 1956). They outline a definite unit-area for collecting the macroinvertebrates within the area. They are designed to be placed by hand onto or in some cases into sand, gravel, or rubble substrate types (usually in riffle/run areas) in shallow streams, or shallow areas of rivers. The drift net sampler (Fig. 8F) is a qualitative and quantitative collecting device used to capture drifting organisms in flowing waters. It differs from the other net type samplers in that it collects from a unit volume of water rather than from a unit area of bottom.

5.7.4 Significance and Use of Stream-Net Samplers

5.7.4.1 The significance of using stream-net samplers is to collect macrobenthos inhabiting a wide range of habitat types from shallow flowing streams or shallow areas in rivers. The stream-net devices (Surber, portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers) are unit area samplers used for collecting benthic organisms in certain types of substrates. They may be used to obtain estimates of the standing crop, for example, biomass, number of individuals and number of taxa of benthic macroinvertebrates per unit area of stream bottom. Efficiency of the sampler depends on the experience and ability of the user. Drift net samplers are designed to collect emigrating or dislodged benthic macroinvertebrates inhabiting all substrate types that either actively or passively enter the water column in flowing streams and rivers and is used to determine drift density and drift rate.

5.7.5 Description of Surber Type Samplers

5.7.5.1 The Surber sampler consists of two 30.5-cm frames, hinged together; one frame rests on the substrate, the other remains upright and holds the nylon net. The sampler is positioned with its net mouth open, facing upstream. When in use, the two frames are locked at right angles, one frame marking off the area of substrate to be sampled and the other frame supporting a net to strain out organisms washed into it from the sample area.

5.7.5.2 Modification of the Surber sampler to overcome some of the limitations of its use (for example, loss of organisms due to backwash) has resulted in the design and construction of a number of related sampling devices, such as the four-sided (enclosed) portable invertebrate box sampler, the cylindrical Hess sampler, the cylindrical Hess stream bottom sampler, and the cylindrical stream-bed fauna sampler. These devices sample 0.1 m^2.

5.7.5.3 Operation of the portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers are similar to the Surber sampler.

5.7.5.4 The net used to collect macroinvertebrates can vary in mesh size, length, taper, and material, for example, canvas, taffeta, or

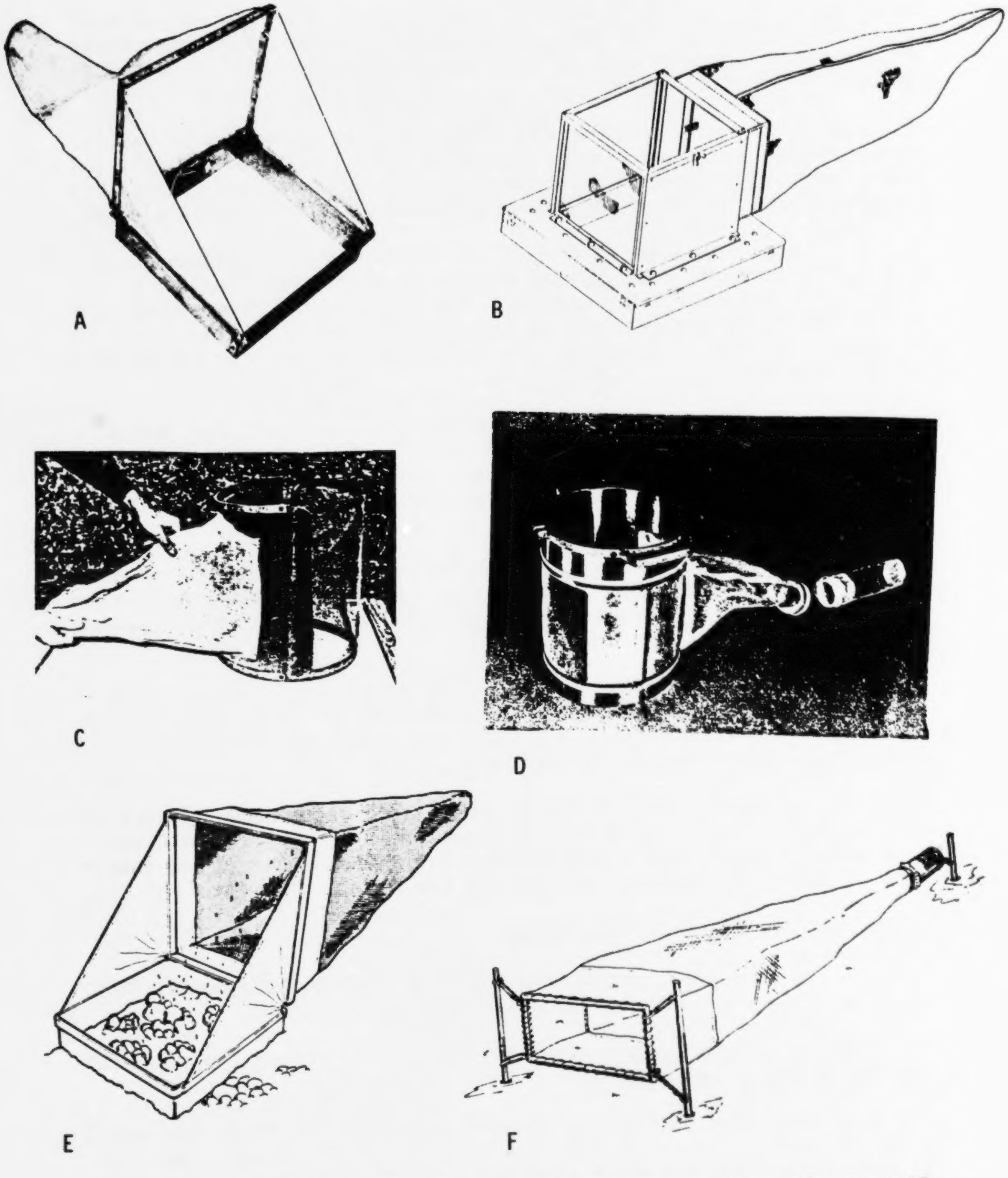

Figure 8. Stream-Net Samplers: (A) Surber sampler; (B) Portable invertebrate box sampler; (C) Hess sampler; (D) Hess stream bottom sampler; (E) Stream-bed fauna sampler (F) Drift net

nylon monofilament. It is usually made of nylon, and a variety of mesh sizes is available. The mesh size used will depend on the objectives of the study. A mesh size of 0.35 mm, for example, will retain most instars of aquatic insects.

5.7.5.6 While a smaller mesh size might increase the number of smaller invertebrates and young instars collected, it will clog more easily and exert more resistance to the current than a larger mesh, possibly resulting in a loss of organisms due to backwashing from the sample net.

5.7.5.7 The polyester foam base of the portable invertebrate box sampler conforms to a variety of substrates to prevent the loss of organisms from beneath the sampler. The Hess, Hess stream bottom, and stream-bed fauna samplers can be "turned" into most sediment types to a depth of several centimeters. The Surber sampler rests on the surface of most sediments.

5.7.5.8 When sampling is completed, the net of the portable invertebrate box sampler slides out for cleaning or exchange with a different net. Hess-type samplers may have a mason jar ring and an adapter with a fixed or removable cloth net bucket. Some of the stream-net samplers have fixed nets.

5.7.5.9 These samplers cannot be used as efficiently in still or deep water of more than 30.48 cm (1-ft) depth. If the water depth is greater than 30.48 cm (1-ft), benthic organisms may wash over the top of the net rather than into it.

5.7.5.10 While there can be large sampling errors associated with their use by an inexperienced operator, these samplers can provide data which are precise and comparable if they are used consistently by one experienced person in similar habitats.

5.7.5.11 If the water velocity is very great, resistance provided by the small mesh of the net or debris washed into it, or both, may result in a backwashing effect that washes benthic organisms out of the sample area of the Surber sampler or over the top of the other samplers.

5.7.6 General Operating Procedures

5.7.6.1 Position these samplers securely on the substrate, parallel to the flow of the water, with the net pointing downstream.

5.7.6.2 The samplers are brought down quickly to reduce the escape of rapidly moving organisms.

5.7.6.3 There should be no gaps under the edges of the frame that would allow for washing of water under the net and loss of benthic organisms. Eliminate gaps that may occur along the edge of the Surber sampler frame by careful shifting of rocks and gravel along the outside edge of the sampler. This is also true of the cylindrical-type samplers if they are on rubble substrate that makes turning into the bottom difficult. The portable invertebrate box sampler polyester foam pad can conform to a relief of 7.6 cm (3 in.).

5.7.6.4 Take care not to disturb the substrate upstream from the sampler, to avoid excessive drift into the sampler from outside the sample area.

5.7.6.5 Once the sampler is positioned on the stream bottom, it should be maintained in position during sampling so that the area delineated remains constant.

5.7.6.6 Hold the Surber sampler with one hand or brace with the knees from behind. The Hess, Hess stream bottom, and stream-bed fauna samplers, and the portable invertebrate box samplers can be held with one hand or braced with the knees from the sides. The portable invertebrate box sampler also can be sat upon for convenience while sampling; this provides the collector with a stable sampling platform that allows maximum manipulation of the substrate with little sampler movement.

5.7.6.7 Heavy gloves should be required when handling dangerous debris; for example, glass or other sharp objects present in the sediment.

5.7.6.8 Turn over and examine carefully all rocks and large stones and rub carefully in front of the net with the hands or a soft brush to dislodge the organisms and pupal cases, etc. clinging to them before discarding. Scrape attached algae, insect cases, etc., from the stones into the sample net.

5.7.6.9 Wash larger components of the substrate within the enclosure with stream water; water flowing through the sampler should carry dislodged organisms into the net.

5.7.6.10 Stir the remaining gravel and sand vigorously with the hands to a depth of 10 cm (4.0 in.) where applicable, depending upon the substrate, to dislodge bottom-dwelling organisms.

5.7.6.11 It may be necessary to hand pick some of the heavier mussels and snails that are not carried into the net by the current.

5.7.6.12 Remove the sample by inverting the net (or washing out sample bucket, if applicable) into the sample container (wide-mouthed jar) with 10% buffered formalin fixative or 70-80% ethanol.

5.7.6.13 Examine the net carefully for small organisms clinging to the mesh, and remove them (preferably with forceps to avoid damage) for inclusion in the sample.

5.7.6.14 Rinse the sampler net after each use.

5.8 Drift Nets

5.8.1 Significance and Use of Drift Nets

5.8.1.1 Macroinvertebrate drift is a normal feature of flowing waters (Brittain and Eikeland, 1988). Drift of organisms may be used to assess environmental stress or pollution in some situations. Stress, fluctuations in water level, changes in light intensity, and changes in temperature are the basic factors that influence the extent of macroinvertebrate drift.

5.8.1.2 One source of drifting macroinvertebrates is the immature insects in the final stages of metamorphosis that actively seek to reach the water surface where emergence to the adult stage occurs. Regular periodic downstream drift rate of immature insects and other macroinvertebrate fauna in slow-moving streams or rivers is markedly reduced in comparison to lotic habitats with rapidly flowing water.

5.8.2.3 Drift insects are about evenly distributed at all levels in a stream, but in large rivers drift is more abundant near the bottom in the shore-line zone.

5.8.2.4 It is generally found that there are pulses of drift organisms that move from top to bottom of the water column, at least during periods of low flow.

5.8.2.5 Drift collections can be used to determine drift density, rate, and periodicity of drift organisms, and interesting aspects of the organisms' life histories, for example, period of transformation.

5.8.2.6 Drift nets are useful for collecting macroinvertebrates that actively or passively enter the water column or that are dislodged from the substrate; naturally or by stress. They are particularly well-suited for synoptic surveys because they are light weight and easily transported.

5.8.2.7 The first step in interpreting drift data is to determine the respective contributions of constant, behavioral, and catastrophic drift to the samples being analyzed.

5.8.2.8 Only constant and behavioral drift are usually utilized in a synoptic survey, but catastrophic drift is extremely important in testing for recent discharges of toxic materials.

5.8.2.9 Bear in mind that the drift density may not be a function of the total bottom population density or of production; however, species composition of the drift is useful as an index of species composition of the benthos.

5.8.2.10 Density and composition of invertebrate drift are influenced by many factors that also must be considered when interpreting the data, including stage of life cycle, weather, time of day, light intensity, population density, temperature, turbidity, water level fluctuation, season, current velocity, growth rate, photoperiod, and proximity to tributary streams.

5.8.2.11 In an enriched stream there is usually a marked increase in total numbers and biomass of drifting organisms as the stream becomes more polluted. Intolerant forms decrease and pollution tolerant forms increase proportional to changing water quality.

5.8.2.12 Thousands of organisms, including larvae of stoneflies, mayflies, caddisflies, and midges and other Diptera, may be collected in a sampling period of only a few hours.

5.8.2.13 The drift net efficiently collects organisms originating from all types of substrates upstream and a wide spectrum of microhabitats in lotic (flowing) waters.

5.8.2.14 The device is restricted to flowing rivers or streams with a current velocity of more than 0.05 m/s.

5.8.3 Advantages of Using Drift Nets

5.8.3.1 A benthic sample shows only which taxa were existing in the particular area (usually some fraction of a square meter, etc.) that was sampled. The great variation among benthic samples, even in a limited area, illustrates the necessity of several samples and the influence of selecting the collecting stations. One drift sample might be adequate for collecting the majority of invertebrate taxa in a stream reach, whereas a large number of benthic samples would be needed to cover the variety of bottom habitats even in an uniform reach of the stream.

5.8.3.2 Quantitative benthic sampling is seldom extended to include stream banks, organic substrates (logs, etc.), and areas of dense vegetation. The drift net collects organisms from all these areas.

5.8.3.3 Drift net collections often require much less sorting work than a series of grab samples. Drift samples do not require the laborious, time-consuming job of washing out silts, clays, and other materials and of sorting and picking through much of the debris for the organisms in the samples.

5.8.3.4 Nets are light-weight and easy to set up in a stream and usually yield a light-weight sample free from most debris. Benthic sampling in flowing water often procures samples heavy with inorganic materials.

5.8.3.6 A drift net is inexpensive to construct, whereas bottom samplers are often costly and more than one kind may be required to adequately sample the multiple habitat types present in a stream or river.

5.8.4 Limitations of Use of Drift Nets

5.8.4.1 Certain aquatic organisms enter the drift only sporadically and might be missed even though common in the benthos.

5.8.4.2 The relative abundance of macroinvertebrates in a drift sample often differs significantly from their "relative" abundance on the stream bottom.

5.8.4.3 A slight current is necessary if a drift collection is to be taken (greater than 0.05 m/s).

5.8.4.4 Most species drift more abundantly at night, so that the best collections are usually taken in the dark. Time of sampling depends on the purpose of the study. Day samples are usually adequate for showing effects of pollution on the stream reach.

5.8.4.5 There is a waiting period while the drifting organisms accumulate in the net, but not as long as with using artificial substrates.

5.8.4.6 Tree leaves in the autumn, floating and anchor ice in the winter, and heavy debris (logs) during floods may interfere with drift net collecting and make processing difficult.

5.8.4.7 The abundance and composition of drift changes, daily, hourly, or seasonally and might prevent direct comparison of collections taken at different times. At times certain life stages of an organism might not be fairly represented in the drift. The same holds true for other types of sampling.

5.8.4.8 Drift collections give little precise habitat information for individual organisms, since the exact source of the individual is not known.

5.8.4.9 Collections of drift, with the organisms originating an indefinite distance above the collecting site, may not show local or temporary deleterious effects imposed on an aquatic community, whereas bottom samples might reveal the destruction or reduction of benthos in a small area. Studies have shown that most drift organisms originate from only several meters upstream from the nets (Elliott, 1967).

5.8.5 Description of Drift Nets

5.8.5.1 The typical drift net consists of a bag of nylon or nylon monofilament. The drift net generally preferred is the simple rectangular net which is light-weight, easy to install, and gives an adequate sample of the drifting macroinvertebrates. The U.S. Standard No. 30 (0.595-mm mesh openings) net is often used for collecting macroinvertebrates.

5.8.5.2 Drift nets vary in size, but the type recommended for use in water pollution surveys or other ecological assessments has an upstream opening of 15 by 30 cm, and the collection bag is 1.3 m long. A variety of mesh sizes is available, and mesh size should be selected based on the objectives of the study; the finer the mesh, the more organisms (instars) will be collected.

5.8.5.3 The frame typically consists of a 0.045-m^2 (15 by 30-cm) brass rod structure anchored into the stream bed by a pair of steel rods.

5.8.5.4 Drift nets are anchored in the stream by driving 1/2-in. steel rods into the stream bottom or mounting the rods in concrete slabs that are weighted down with stones. Use cable clamps to secure the nets to the rods.

5.8.5.5 The drift net frame can be fitted anteriorly with a mouth reducing rectangular plexiglass enclosure (Rutter and Ettinger 1977) to increase filtration efficiency and volume of water passing through the net.

5.8.5.6 Alternatives to the typical drift net include the waterwheel drift sampler (Pearson and Kramer, 1969) which might be useful in large rivers or streams with slow flow which can be reached by automobile.

5.8.5.7 An automatic drift sampler (Muller, 1965) can be constructed that eliminates the need of an attendant at the sampling site during collection of as many as eight consecutive samples.

5.8.5.8 A modified emergence-trap drift sampler (Mundie, 1964; Cushing, 1964) is useful in streams with extremely high drift, where water is very turbid, or where a long sampling period is desired without clogging.

5.8.5.9 The average volume of water passing through the net is determined by measuring the water velocity at the mouth of the drift net with a current meter at the beginning of the sampling period and at the end of the sampling period using the average, and recording the total time the drift net is set in the water column. Results are expressed as numbers per cm^3 of water passing through the net.

5.8.5.10 The efficiency of the net is determined by the simultaneous measurement of the water velocity passing by the set drift net.

5.8.6 General Operating Procedures

5.8.6.1 Because the performance and sampling efficiency of a drift net sampler varies with local stream conditions, seasonal changes, and water level, make a preliminary test before the start of regular drift sampling in order to determine the best sampling stations, best sampling interval, number of nets needed, mesh size, and best sampling depth.

5.8.6.2 For synoptic surveys, one net set above each of the major areas of population concentrations is usually adequate; but for definitive studies a minimum of two drift nets should be set at each station so that drift from above a pollution source, drift from the polluted reach, and drift from the zone of clean water downstream from the recovery zone can be compared.

5.8.6.3 Take into consideration the fact that the drift net will collect drifting organisms that may have entered the drift from an indefinite distance upstream or a tributary stream. Nets located 80 to 100 m below the effluent will generally sample the polluted reach efficiently. A drift net below a riffle collects more animals than one below a pool.

5.8.6.4 For definitive studies, install four nets at each station - two about 25 cm from the bottom and two about 10 cm below the surface in water not exceeding 3 m in depth.

5.8.6.5 If the objective of the study is to relate pupal exuviae to pollution, or to collect terrestrial organisms that may float on the surface, then extend one net slightly above the surface.

5.8.6.6 Ideally, collect 24-h drift samples; but this is usually not practicable unless one resorts to the use of a water-wheel, automatic drift sampler, or a modified drift sampler with a restricted opening to solve the clogging problem or by changing the nets at regular intervals.

5.8.6.7 Although the sampling interval will vary with time of day, current velocity, density of drift organisms, and floating debris, collect 1-3 hours daytime drift samples when either a 24-h or overnight sampling period is not prudent.

5.8.6.8 Drift nets have also been used from small boats in large rivers (Rutter and Ettinger, 1977).

5.8.6.9 Because the size of the catch varies as the flow of water through the net varies, it is necessary to measure the current velocity at the entrance of each net at the beginning and end of each sampling period so that the catch can be converted into number of organisms per volume of water flowing through the net.

5.8.6.10 At the end of the specified sampling period, remove the net from the water by loosening the cable clamps and raising the net over the top of the steel rods, taking care not to disturb the bottom upstream of the net.

5.8.6.11 Concentrate the material in the net in one corner by swishing up and down in the water and then wash into a bucket half-filled with water. Then sieve and handle the sample in the regular manner.

5.8.6.12 Subdividing the sample substantially reduces analysis time with large samples (Waters, 1969a and USEPA, 1973).

5.8.6.13 Reporting data as numbers of individuals per net is meaningless because no two drift net samples are collected under exactly the same conditions of current velocity, stream discharge, and sampling interval. Conversion equations and other statistical aspects of drift sampling are given by Elliott (1970). An equation for converting the data to number per 100 m^3 of water flow is:

$$X = 100a/bdc$$

where:
X = number of organisms per 100 m^3,
a = number of organisms in the net (density)
b = number of minutes of the sampling interval,
c = current velocity, m/min, and
d = area of the net opening in m^2.

5.9 Artificial Substrate Samplers

5.9.1 Artificial substrate samplers are devices made of natural or artificial materials of various composition and configuration that are placed in water for a predetermined period of exposure and depth for the colonization of indigenous macroinvertebrate communities. They are used in obtaining qualitative and quantitative samples of macroinvertebrates in rivers, streams, lakes, and reservoirs.

5.9.2 Artificial substrate sampling can effectively augment bottom substrate sampling because many of the physical variables encountered in bottom sampling are minimized (e.g., variable depth and light penetration, temperature differences, and substrate types).

5.9.3 Samples usually contain negligible amounts of extraneous material, permitting quick laboratory processing.

5.9.4 Selecting Artificial Substrate Samplers

5.9.4.1 Table 5 summarizes criteria for selecting artificial substrate samplers.

TABLE 5. SUMMARY CRITERIA FOR ARTIFICIAL SUBSTRATE SAMPLERS

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1. Multiplate (Modified Hester-Dendy) Sampler

   A. Habitats and Substrates Sampled: All types of habitats in rivers, streams, lakes and reservoirs; not efficient in wetlands; uses hardboard or porcelain substrate.

   B. Effectiveness of the Device: Colonization depends on type of substrate; selective for certain types of organisms; three replicates considered adequate.

   C. Advantages: Excellent for water quality monitoring; uniform substrate type; high level of precision; samples contain negligible amount of debris; provides habitats of known area for a known time at a known depth.

TABLE 5. SUMMARY CRITERIA FOR ARTIFICIAL SUBSTRATE SAMPLERS (Continued)

D. Limitations: Requires trip for installation and trip for collection; subject to vandalism; biased for aquatic insects; need to use caution in reuse of plates that may have been contaminated with toxicants, oil, etc.; may require additional weight for stability; up to eight weeks wait for results.

Selected Literature: APHA, 1989; Beck et al., 1973; Beckett and Miller, 1982; Cairns, 1982; Flannagan and Rosenberg, 1982; Fullner, 1971; Greeson et al., 1977; Hall, 1982; Harrold, 1978; Hester and Dendy, 1962; Hellawell, 1978; Jacobi, 1971; Mason et al., 1973; McConville, 1975; McDaniel, 1974; Merritt and Cummins, 1984; Ohio EPA, 1987; Rosenberg and Resh, 1982; USEPA, 1973; Wefring and Teed, 1980.

2. Basket Sampler

A. Habitats and Substrates Sampled: All types of habitats in rivers, streams, lakes and reservoirs; may be used in areas where other methods are not feasible; not efficient for sampling in wetlands.

B. Effectiveness of the Device: Colonization depends on type of artificial substrate used in the basket (rocks, 3M Conservation Webbing, etc.); selective of certain types of fauna; three replicates considered adequate.

C. Advantages: Excellent for water quality monitoring; uniform substrate type at each station for better comparison and high level of precision; gives quantitatively comparable data; samples contain negligible amounts of debris; does not require additional weight for stability; samples a known area at a known depth for a known exposure time.

D. Limitations: Require trip for installation and another for collection; biased for insects; samplers and floats often difficult to anchor; may be navigation hazard; susceptible to vandalism; records only biotic community present during exposure period; no measure of past conditions; size and texture of limestone substrates may vary from study to study; up to eight weeks wait for results.

Selected Literature: Anderson and Mason, 1968; APHA, 1989; Benfield et al., 1974; Bergensen and Galat, 1975; Bull, 1968; Cairns, 1982; Flannagan and Rosenberg, 1982; Hall, 1982; Hanson, 1965; Hellawell, 1978; Leopold, 1970; Lium, 1974; Mason et al., 1967, 1973; Merritt and Cummins, 1984; Newlon and Rabe, 1977; Rabeni and Gibbs, 1978; Rabeni et al., 1985; Rosenberg and Resh, 1982; USEPA, 1973; Voshell and Simmons, 1977; Zillich, 1967.

5.9.5 Significance and Use of Artificial Substrate Samplers

5.9.5.1 Multiple-plate and basket samplers (Figure 9A-F) are usually colonized by a wide variety of invertebrates which have some means of mobility (active or passive) that are borne in the current. The organisms that colonize the artificial substrates are primarily aquatic insects, aquatic oligochaetes, crustaceans, cnidarians, turbellarians, bryozoans, and mollusks. The colonization of these organisms should be relatively equal in similar habitats and reflect the capacity of the water to support aquatic life. Although these samplers may exclude certain mollusks or worms, they collect a sufficient diversity of benthic fauna to be useful in assessing water quality.

5.9.5.2 Recovery techniques are critical for insuring collection of all organisms retained on the sampler.

5.9.5.3 Uniform substrate type reduces the effects of substrate differences.

5.9.5.4 Optimum time for substrate colonization is 6 weeks for most water in the United States.

5.9.5.5 Quantitatively comparable data can be obtained in environments from which it is virtually impossible to obtain samples with conventional devices.

5.9.6 Description of Multiple-Plate Samplers

5.9.6.1 Multiple-plate samplers consist of standardized, reproducible artificial substrate surfaces for colonization by aquatic organisms. Their uniform shape and texture compared to natural substrates greatly simplifies the problem of sampling. The sampler is constructed from readily available materials.

5.9.6.2 The modified multiple-plate sampler (Fig. 9A,B) is constructed of 0.125 in (0.3 cm) tempered hardboard or ceramic material with 3 in (7.6 cm) round or square plates and 1 in (2.5 cm) round spacers that have 5/8 in holes drilled in the center (Fullner, 1971). The plates are separated by spacers on a 0.25 in (0.63 cm) diameter eyebolt, held in place by a nut at the top and bottom. A total of 14 large plates and 24 spacers are used in each sampler. The top nine plates are each separated by a single spacer, plates 9 and 10 are separated by two spacers, plates 11 and 12 are separated by three spacers, and plates 13 and 14 are separated by four spacers. The hardboard sampler is about 5.5 in (14 cm) long, 3 in (7.6 cm) diameter, exposes approximately 1,160 cm^2 (.116 m^2) of surface area for the attachment of organisms, and weighs about 1 lb (0.45 kg). The ceramic sampler is 6.5 in. long and weighs 2.2 lbs (1 kg). The ceramic plates can be chemically cleaned, oven dried and reused indefinitely as they are stable and unaffected by long-term immersion in water. The sampler will not warp with time; therefore, the spacings between plates do not change, assuring replicate and efficient sampling. Each sampler is supplied with a 6 m (20′) long nylon suspension rope. The total weight is 1 Kg (2.2 lbs.). Sturdy

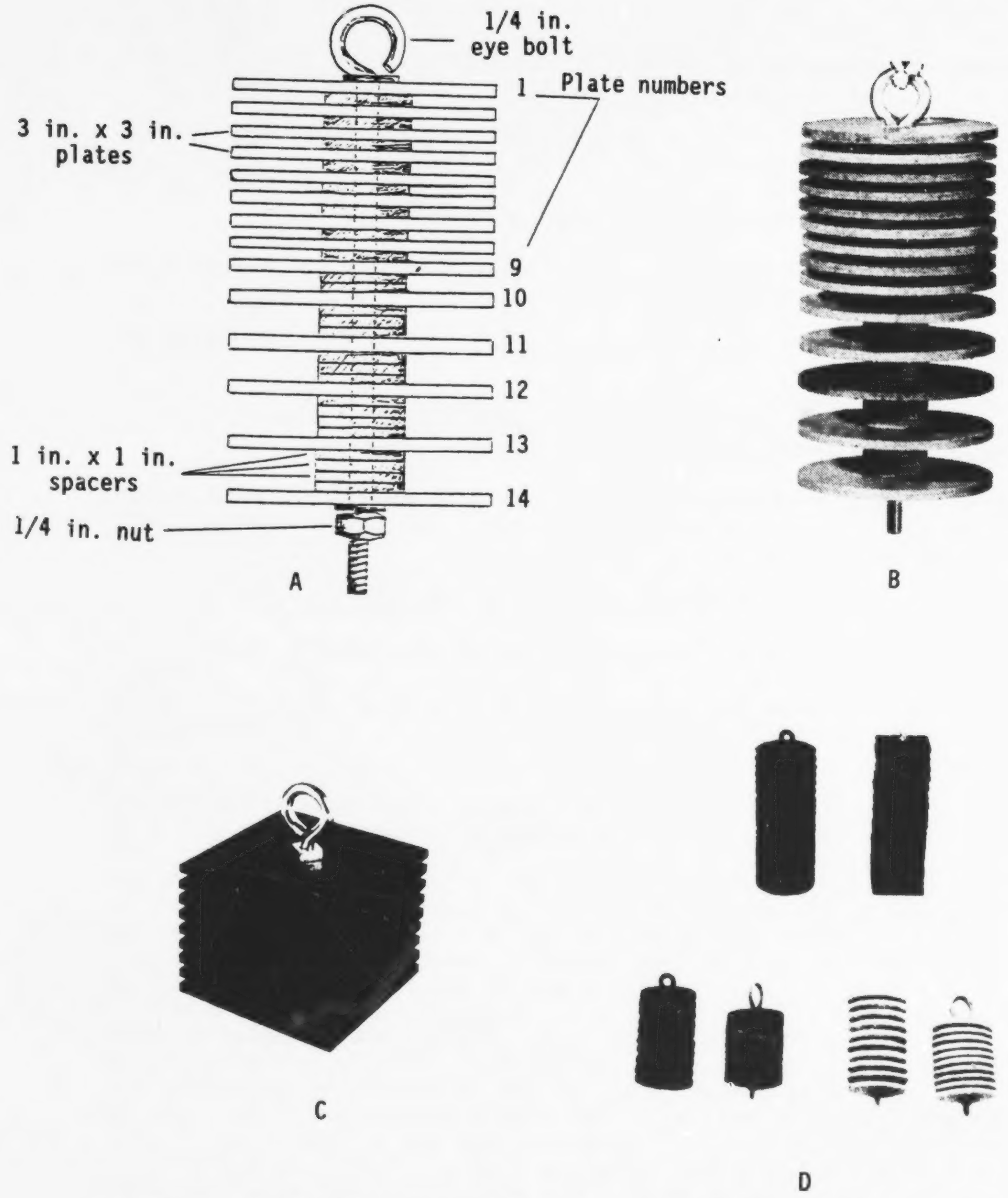
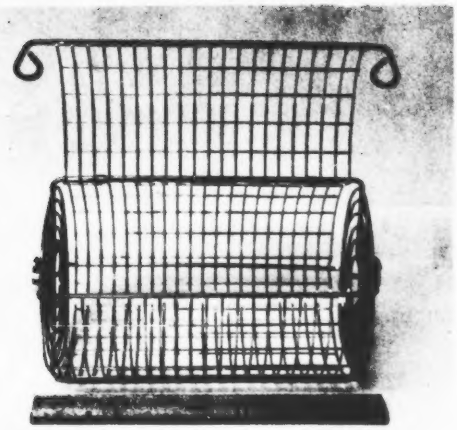
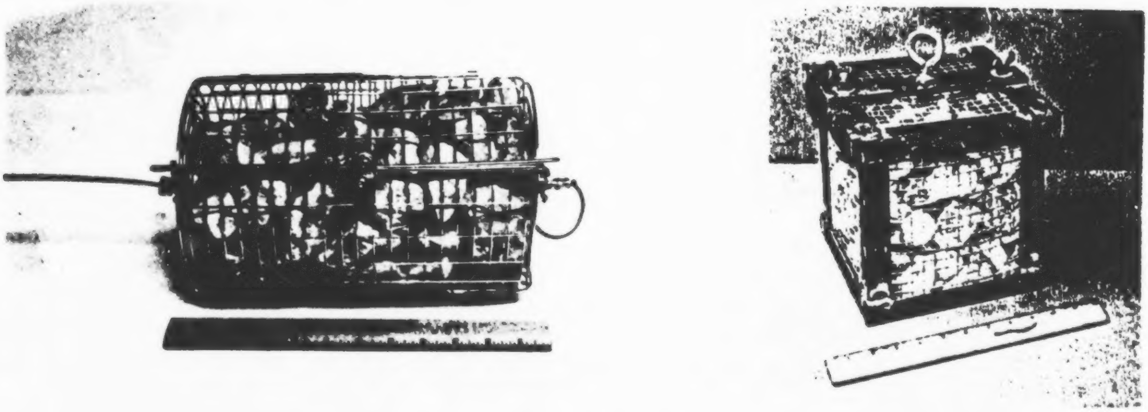

1/4 in.
eye bolt
1
Plate numbers
3 in. x 3 in.
plates
9
10
11
12
13
1 in. x 1 in.
spacers
14
1/4 in. nut
A
B
C
D

Figure 9. Artificial Substrate Samplers: (A) Schematic drawing of multiplate Sampler; (B) Typical round multiplate type; (C) Original Hester-Dendy multiplate, square design; (D) Jumbo and standard hardboard and porcelain multiplate designs



E



F

Figure 9. Artificial Substrate Samplers: (E) Barbecue basket; (F) Basket samplers, cylindrical and square types

wire stakes for holding the sampler above the riverbed are recommended accessories.

5.9.6.3 When the samplers are suspended from the eyebolt, whether in strong currents or not, a 5 lb weight, such as a brick, is attached by .6 m wire to a 1/4 in turnbuckle. The turnbuckle is screwed tightly onto the shank of the multiplate eyebolt. The weight serves to stabilize the sampler and to lessen undue disturbance to the organisms. Upon retrieval, the weight is gently cut free before the sampler is bagged. Care should be taken not to reuse samplers exposed to oils and chemicals that may inhibit colonization during the next sampling period. Due to its cylindrical configuration, the sampler fits a wide mouth container for shipping and storage purposes. The sampler is inexpensive, compact, and light weight which are valuable attributes in water quality surveys.

5.9.7 Description of a Basket Sampler

5.9.7.1 The typical type of basket sampler (Fig. 9E) used is the one described by Mason et al. (1967). It is a cylindrical "barbecue" basket 11 in (28 cm) long and 7 in (17.8 cm) in diameter and is filled with approximately 17 lbs (7.7 kg) of natural rocks that vary from 1 to 3 in (2.5 to 7.6 cm) in diameter. A hinged door on the side allows access to the contents. An estimated 3.2 square ft (0.3 sq. m) of surface area is provided for colonization by macroinvertebrates. A 1/8 inch wire cable is passed through the long axis of the basket; one end is fastened with a cable clamp, and the other end is attached to a 5 gallon metal container filled with polyurethane foam used as a float. A 3/8 inch steel rod that is threaded at each end is passed through the long axis of the float and fastened at each end by nuts. Three inch long 1-1/8 by 1/8 inch strap iron secured on the rods by nuts serves as swivels at each end. The wire cable used to suspend the basket is attached to the swivels by holes drilled for that purpose. The float can be attached to a stationary structure or the basket can be anchored to the bottom in shallow water. The rugged construction of this particular basket sampler is heavy enough to resist movement by most water currents. In using the basket as a method of collecting macroinvertebrates, special consideration should be given to the types of substrates placed within the basket. Substrates tested have varied from limestone, tin cans, concrete cones, #200 3M Conservation Webbing (3M Corporation, St. Paul, MN), and porcelain spheres. Since each type of substrate will result in a different species diversity, the type of substrate used should be determined by the study objectives, weighing the advantages and disadvantages of each substrate type. For most investigations, a basket filled with 30 5-8 cm diameter rocks or rock-like material is recommended.

5.9.8 Precautions

5.9.8.1 Physical factors such as stream velocity and installation depth may variably affect degree of colonization.

5.9.8.2 The sampling method is selective for drifting organisms and for those which preferentially attach to hard surfaces.

5.9.8.3 Recovery techniques are critical for insuring collection of all organisms retained on the sampler.

5.9.8.4 Samplers are vulnerable to vandalism and often lost.

5.9.8.5 Caution should be exercised in reuse of samplers that may be subjected to contamination by toxicants, oils, etc.

5.9.8.6 The sampler provides no measure of the biota and the condition of the natural substrate at a station or of the effect of pollution on that substrate.

5.9.8.7 Sampler and floats must be anchored or fixed in place. This is sometimes difficult, and they may present a navigation hazard.

5.9.8.8 The sampler only records the community that develops during the sampling period, thus reducing the value of the collected fauna as indicators of prior conditions.

5.9.9 General Operating Procedures

5.9.9.1 Artificial substrate samplers are usually positioned in the euphotic zone of good light penetration (one to three feet, or .3-.9 m) for maximum abundance and diversity of macroinvertebrates (Mason, et al. 1973). Optimum time for substrate colonization is six weeks for most types of water in the United States. For uniformity of depth, suspend sampler from floats on 1/8 in. or 3.2 mm steel cable. If water fluctuation is not expected during sampling period, the samplers may be suspended from stationary objects. If vandalism is a problem, use subsurface floats or place sampler on supports placed on the bottom. Regardless of installation technique, use uniform procedures (e.g., same exposure period, sunlight, current velocity and habitat type). At shallow water stations (less than 1.2 m deep), install samplers so that the exposure occurs midway in the water column at low flow. If the samplers are installed in July when the water depth is about four feet and the August average low flow is two feet, the correct installation depth in July is one foot above the bottom. The sampler will receive sunlight at optimum depth (one foot) and will not be exposed to air anytime during the sampling period. Care should be exercised not to allow the samplers to touch bottom which may permit siltation, thereby, increasing the sampling error. In shallow streams with sheet rock bottoms, artificial substrate samplers are secured to 3/8 in. (.95 cm) steel rods that are driven into the substrate or secured to rods that are mounted on low, flat rectangular blocks (Hilsenhoff, 1969). These must, however, be securely anchored to the rock bottom to avoid loss during floods.

5.9.9.2 Artificial substrate samplers can be attached to floats, cement structures, a weight, or a rod driven into the stream-bed or lake-bed.

At least two or three samplers should be installed at each collecting site. Leave the samplers in place for at least 6 weeks to allow for organism colonization. The exposure time should be consistent among sites during the study. If study time limitations reduce this period, the data must be evaluated with caution, and in no case should data be compared from samplers exposed for different time periods.

5.9.9.3 The samplers may be installed in pools or riffles/runs suspended below the water surface. Make the collections as representative of the reach as possible by insuring that the samplers are not to close to the bank. In streams up to a few meters in width, install the devices about midstream. In larger streams install the devices at about one-quarter of the total width from the nearest bank.

5.9.9.4 To minimize losses of animals when retrieving multiplate and basket samplers, approach from downstream, lift the sampler quickly and place the entire sampler in a polyethylene jug or bag containing 10% formalin or 70-80% ethanol. Once the sampler is touched it must be removed from the water at once or many of the animals will leave the sampler. If the sampler must be disturbed during the recovery process so that it cannot be lifted straight up out of the water, a net should be used to enclose the sampler before it is disturbed.

5.9.9.5 The organisms can be removed in the field by disassembling the sampler in a tub or bucket partially filled with water and scrubbing the rocks or plates with a soft-bristle brush to remove clinging organisms. Pour the contents of the bucket through a No. 30 or 60 sieve and wash the contents of the sieve into a jar and preserve with 10% formalin or 70-80% ethanol. If the organisms are not removed in the field, place the sampler and the detached portion of sample into a wide-mouth container or sturdy plastic bag containing preservative for transporting to the laboratory. Label the sample with the location, habitat, date, and time of collection. The exposed multiplate sampler can be taken to the laboratory where the plates are removed from the bolt and cleaned with a soft-bristled brush. The basket samplers are usually disassembled in the field; however, they can be taken to the laboratory and disassembled if placed in preservative in a water-tight container.

5.9.9.6 Cleaned samplers can be reused unless there is reason to believe that contamination by toxicants (e.g., chemicals or oils) has occurred. These substances may be toxic to the macroinvertebrates or may inhibit colonization. Do not reuse hardboard, porcelain plates, or any other substrate that have been exposed to preservatives. Clean the multiple-plates before reassembly and use.

5.10 **Coring Devices**

5.10.1 Included in this category are single and multiple-head coring devices, tubular inverting devices, and open-ended stovepipe devices.

5.10.2 Selecting Coring Devices

5.10.2.1 Table 6 summarizes criteria for selecting coring devices

TABLE 6. SUMMARY CRITERIA OF CORING DEVICES

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1. KB Core Sampler

   A. Habitats and Substrates Sampled: Freshwater rivers,lakes, estuaries; soft sediments only, 40% silty clay.

   B. Effectiveness of the Device: Permits analysis of stratification in quantitative and qualitative samples; uses 5.08 cm (2 inch) pipe core tube; used in shallow to medium shallow water up to 30.5 m (100 feet) or deeper.

   C. Advantages: Samples a variety of substrates up to harder types; sampling tube can be modified for various diameters up to 100 cm^2 substrate surface; least disturbance to water/bottom interface; standard and heavy models available; wide variety of core tubes. liner tubes, core catchers, and nosepieces.

   D. Limitations: Gravity operated; samples limited surface area; standard KB core sampler head, without core tube weights approximately 8 kg (18 pounds), but additional weight can be added to sampler; requires boat and powered winch.

2. Ballchek Single and Multiple Tube Core Sampler

   A. Habitats and Substrates Sampled: Same as KB Core Sampler.

   B. Effectiveness of the Device: Samples deep burrowing organisms in soft sediment, particularly effective for sampling oligochaetes; uses 5.08 cm (2 inch) or 7.62 cm (3 inch) pipe core tube; used in shallow or deep waters, 3 m to 183 m (10-600 feet); multiple core sampler weight approximately 38 kg (84 pounds); check valves work automatically, prevent loss of sample.

   C. Advantages: Good penetration in soft sediments; small volume of sample allows for greater number of replicates to be analyzed in a short period of time; single or multiple (four) core tube sampler available; three inch pipe for larger cores and/or deep water lakes and oceans available; wide variety of core tubes, liner tubes, core catchers, and nosepieces.

   D. Limitations: Heavy device, approximately 38 kg, requires boat and winch; gravity operated; does not retain sand unless bronze core retainers are used which require additional weight to insure penetration.

3. Phelger Core Sampler

TABLE 6. SUMMARY CRITERIA OF CORING DEVICES (Continued)

A. Habitats and Substrates Sampled: Same as above core samplers.

B. Effectiveness of the Device: Similar to KB core sampler.

C. Advantages: Similar to KB core sampler.

D. Limitations: Gravity operated or can be messenger operated with a suspension-release device; styles and weights vary among manufacturers, some use interchangeable weights, between 7-35 kg, others use fixed weights up to 41 kg; length core taken varies with substrate texture.

4. Box Core Sampler

A. Habitats and Substrates Sampled: Same as above core samplers, also oceans.

B. Effectiveness of the Device: Same as above core samplers; samples a surface area of 100 cm^2 and a sediment depth of 20 cm.

C. Advantages: Same as above core samplers.

D. Limitations: Same as above core samplers; also deployed from ships or other platforms; diver collected cores are preferred.

5. Hand-Operated Core Samplers

A. Habitats and Substrates Sampled: Same as above core samplers.

B. Effectiveness of the Device: Sampled by hand or by diver.

C. Advantages: Can be used in shallow water. In deep water can be used with a diver, usually a trained biologist, who can collect and recognize substrate and bottom changes to stratify sampling; can be used with extension handles of 5, 10, or 15 feet; used with pipe fitting for driving from a pontoon boat, dock, or bridge.

D. Limitations: Limited area sampled.

Selected Literature: APHA, 1989; Brinkhurst, 1967, 1974; Burton, 1974; Coler and Haynes, 1966; Edmondson and Winberg, 1971; Flannagan, 1970; Gale, 1977; Hamilton _et al_., 1972; Holme, 1964; Holme and McIntyre, 1971; Miller and Bingham, 1987; Poole, 1974; Schwoerbel, 1970.

5.10.2.2 Coring devices can be used at various depths in any substrate that is sufficiently compacted so that an undisturbed sample is retained; however, they are best suited for sampling the relatively homogenous soft sediments, such as clay, silt, or sand of the deeper portions of lakes, reservoirs, and oceans. Because of the small area sampled, data from coring devices are likely to provide very imprecise estimates of the standing crop of macrobenthos.

5.10.2.3 KB type, Ballchek, and Phleger corers (Fig. 10A,B,C) are examples of devices used in shallow and deep water; they depend on gravity to drive them into the sediment. The cores are designed so that they retain the sample as it is withdrawn from the sediment and returned to the surface. Hand corers (Fig. 10D) designed for manual operation are used in shallow water. Sections of the core can be extruded and preserved separately or the entire core can be retained in the tube and processed in the field or laboratory. Intact cores can also be preserved by freezing and processed later.

5.10.2.4 Additional replication with corers is feasible because of the small amount of material per sample that must be handled in the laboratory. Multiple-head corers have been used in an attempt to reduce the field sampling effort that must be expended to collect large series of core samples (Flannagan, 1970).

5.10.2.5 The Dendy inverting sampler (Welch, 1948) is a highly efficient coring-type device used for sampling at depths to 2 or 3 meters in nonvegetated substrates ranging from soft muds through coarse sand. Because of the small surface area sampled, data obtained by this sampler suffer from the same lack of precision (Kajak, 1963) as the coring devices described above. Since the per-sample processing time is reduced, as with the corers, large series of replicates can be collected. The Dendy sampler is highly recommended for use in habitats for which it is suitable.

5.10.2.6 Stovepipe-type devices include the Wilding sampler (Wilding, 1940; APHA, 1989) and any tubular material such as 60-to-75 cm sections of standard 17-cm-diameter stovepipe (Kajak, 1963) or 75-cm sections of 30-cm-diameter aluminum irrigation pipe fitted with handles. In use, the irrigation pipe or commercial stovepipe is manually forced into the substrate, after which the contained vegetation and coarse substrate materials are removed by hand. The remaining materials are repeatedly stirred into suspension, removed with a long-handled dipper, and poured through a wooden-framed floating sieve. Because of the laborious and repetitive process of stirring, dipping, and sieving large volumes of material, the collection of a sample often requires 20 to 30 minutes.

5.10.2.7 The use of stovepipe samplers is limited to standing or slowly moving waters having a maximum depth of less than 60 cm. Since problems relating to depth of sediment penetration, changes in cross-sectional area with depth of penetration, and escapement of organisms are circumvented by stovepipe samplers, they are recommended for quantitative sampling in all shallow-water benthic habitats. They probably

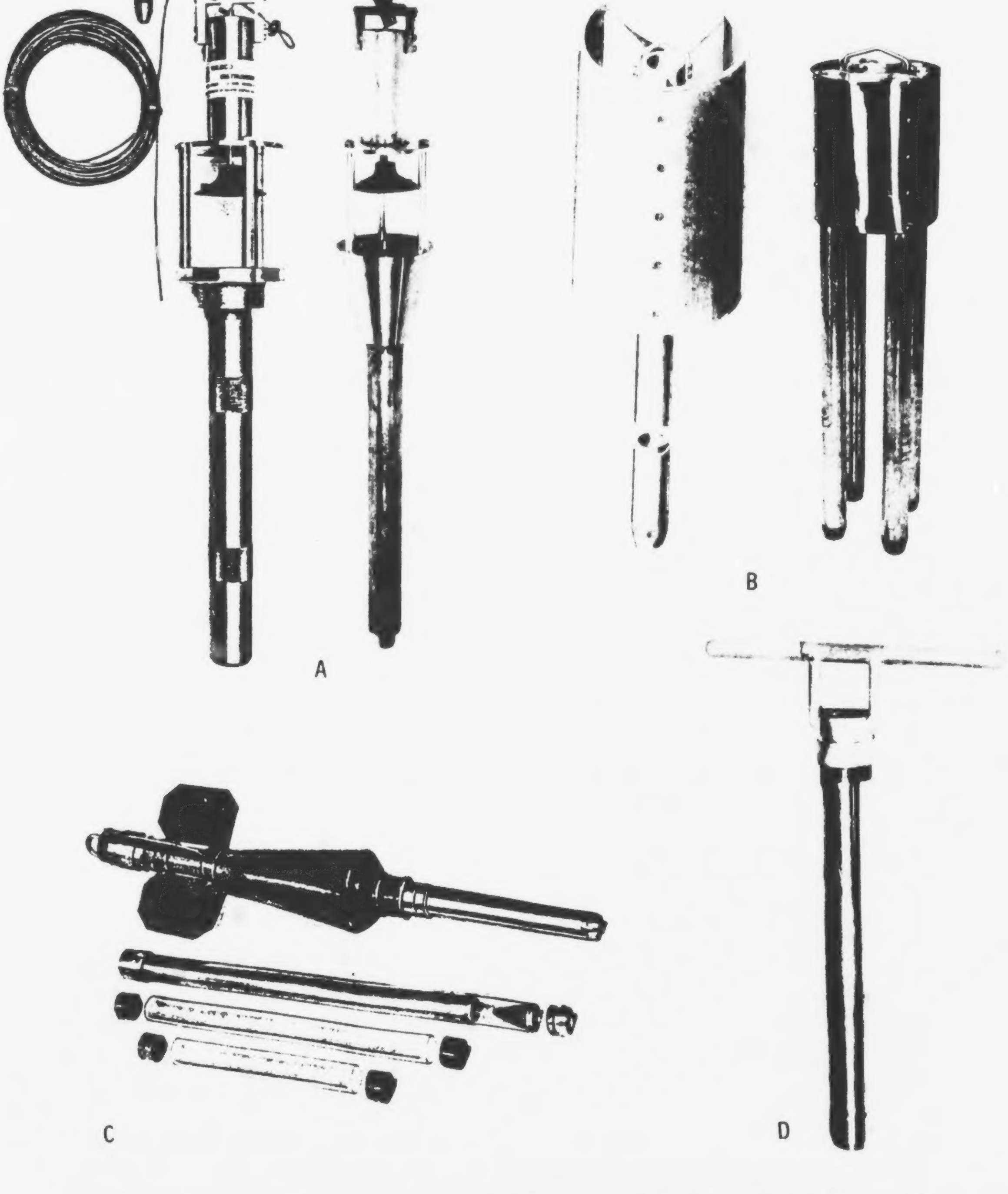

Figure 10. Core Samplers: (A) KB corer, standard and heavy duty; (B) Ballchek corer, single and multiple types; (C) Phleger corer; (D) Hand-operated corer

represent the only quantitative device suitable for sampling shallow-water habitats containing stands of rooted vascular plants and they will collect organisms inhabiting the vegetative substrates as well as those living in sediments.

5.10.2.8 In marine waters benthic macrofauna are generally collected using various box cores deployed from ships or other platforms, or diver collected cores. A box coring device consisting of a rectangular corer having a cutting arm which can seal the sample prior to retraction from the bottom should be used. In order to sample a sufficient number of individuals and species, and to integrate the patchy distribution of fauna, each sample should have a surface area of no less than 100 cm^2 and a sediment depth of at least 20 cm. In sediments having deep, burrowing fauna, a box corer capable of sampling deeper sediment may be needed. In sandier sediments, it may be necessary to substitute a grab sampler for the box corer in order to achieve adequate sediment penetration. Sufficient replicates (usually 3 to 10) should be taken to produce an asymptotic cumulative species curve. Visual inspection of each sample is necessary to insure an undisturbed and adequate amount of sample is collected.

5.11 Frames

5.11.1 For estimating the populations of attached marine organisms on a rocky shore, 0.1 m^2 or 1 m^2 square-shaped metal frames can be used for delineating percent coverage of the colonial forms. At least ten frames should be counted for characterizing the distribution statistically. Samples of the algae and macroinvertebrates should be removed from a measured area for species identification and weighed for biomass determination. It is important to note the attitude of the sampling frame relative to the horizontal and vertical axis in order to relate the data with the zonation patterns. A vertical plane is apt to have a dramatically different species array compared to a horizontal plane even with both being at the same level with the intertidal zone.

5.11.2 Attaching a 35 mm SLR camera to a sampling frame so that the focal distance is fixed is an excellent method for documenting the population present at each sampling site. Species enumeration and percent cover can be estimated from the developed photographs. This method is especially useful for documenting temporal changes at a particular sampling site.

5.11.3 For sampling the infauna of beaches, a 0.1 m^2 square metal frame with a 15 cm lip is useful. The frame can be deliberately thrown near a fixed position (see Section 4.4.3, Systematic Sampling). Stovepipe or large coffee can work very well in most sandy, sandy-mud beaches but have limited use in cobble beaches. All of the substrate is removed and screened in fine-meshed screens. The animals retained are washed or picked from the screens and preserved for later identification and enumeration.

5.11.4 Edged frames (.1 m^2) or corers can be utilized for systematically sampling the substrates around fixed positions on the flats. At least five replicate samples should be collected at each site for statistically delineating the distribution patterns of the infauna populations. The substrate is then washed through fine meshed screens. The invertebrates can be washed or picked from the screens and preserved. Flats represent areas of quiet, low velocity waters with the settling of suspended materials. Flats near pollution sources are good sites to observe the impact of all settled materials, non-toxic and toxic. Some flats are so poorly drained as to require snowshoes or similar devices for walking out to the sampling area. In such areas, it may be easier to sample at high tide from a boat using a conventional benthic grab.

5.12 Rapid Bioassessment Protocols (RBPs) for Macroinvertebrates (see Plafkin et al., 1989 and Section 7, Data Evaluation.)

5.12.1 The methods describe three different protocols (I,II, and III) for use in wadable streams and rivers to determine water quality. The RBPs are considered qualitative and semi-quantitative sampling techniques for assessing the health of benthic macroinvertebrate communities. The protocols consist of three basic components--water quality and physical characteristics, habitat assessment, and biosurvey. The biological assessment involves integrated data analyses of both functional and structural components of the macroinvertebrate communities through the use of metrics. The protocols describe guidelines for a rapid means of detecting water quality and aquatic life impairments and assessing their relative severity. The RBPs are not intended to replace traditional biomonitoring methods but provide an option which may be cost effective. These RBPs work very well as a surveillance tool to prioritize sites for more intensive evaluations (quantitative biological surveys) but are not always comparable to the results obtained with more traditional methods such as artificial substrate samplers or drift nets. The same metrics (RBPs) may be used with these more traditional methods of collection and give qualitative or quantitative results.

5.12.1.1 Protocol I provides for basic qualitative information for a subjective judgment of macroinvertebrate abundance and presence. The method consists of habitat assessment and the collection of macroinvertebrates from all possible habitats. The specimens are identified to orders and counted in the field. The data are used to make a subjective assessment of stream water quality or impairment.

5.12.1.2 Protocol II provides a reasonably reproducible assessment of biological impact and consists of habitat assessment and collecting macroinvertebrates from all available habitats. The specimens are identified to families, and the list of families in a 100-organisms subsample is used in the evaluation. The study is based on established guidelines in scoring parameters, and the stream site would be classified as to water quality or degree of impact and possible cause.

5.12.1.3 The objectives of Protocol III are to assess the biological impact and to establish the basis for trend monitoring of pollution effects over a period of time. The method consists also of specific guidelines for evaluating the habitat assessment parameters and collecting macroinvertebrates from all available habitats. The protocol is similar to Protocol II except that the specimens are identified to the lowest possible taxonomic level (genus, species). The data are categorized into parameters based on taxa richness, biotic index, percent composition, and functional group designations. The classification of stream sites is dependent on established guidelines.

5.13 Ohio EPA Invertebrate Community Index method (ICI) (see Ohio EPA, 1987, 1989)

5.13.1 The ICI semi-quantitative method uses 10 metrics to determine if wadable streams or rivers are polluted using benthic macroinvertebrates. Nine of the 10 metrics are based on multiple plate artificial substrate samples, and one is based on dip net sampling (Ohio EPA, 1987 and 1989). Also, see Section 7, Data Evaluation.

5.14 Standard Qualitative Collection Method (see Lenat, 1988; Eagleson, *et al*., 1990, and NC DEM, 1990 and Section 7, Data Evaluation)

5.14.1 The method emphasizes multiple-habitat sampling, field-picking of samples, and the use of both coarse- and fine-mesh samplers. This standard qualitative method consists of collecting macroinvertebrates in shallow streams, usually less than 1.5 m deep using two kick net samples, three dip net samples (sweeps), one leaf-pack sample, three aufwuchs samples, one sand sample, and visual search collections. The data resulting from this method, especially taxa richness, can be used to assign water quality ratings. The method is applicable for most between-site and/or between-date comparisons. Also, a secondary abbreviated qualitative method (EPT survey) can be used to quickly determine between-site differences in water quality. The number of collections is decreased from 10 samples in the standard quality collections to only four samples: one kick, one sweep, one leaf-pack and visual searches in the abbreviated method.

5.15 Miscellaneous Qualitative Devices

5.15.1 The investigator has an unlimited choice of gear for collecting qualitative samples. Any of the quantitative devices discussed previously, plus hand-held screens, dip nets, sweep nets, kick nets, rakes, tongs, post-hole diggers, bare hands, and forceps can be used for collecting benthic macroinvertebrates from freshwater, estuarine, and marine environments. For deep-water collecting, some of the conventional grabs described earlier and dredges are normally required. In water less than 2 meters deep, a variety of gear may be used for sampling the sediments including long-handled dip nets and post-hole diggers. Collections from vascular plants and filamentous algae may be made with a dip net, common garden rake, potato fork, or oyster tongs. Collections from floating debris and rocks may be made by hand, using forceps to catch the smaller organisms. In shallow streams, short sections of common window screen may be fastened between two poles and held in place at right angles to the water flow to collect organisms dislodged from upstream materials that have been agitated.

5.15.2 Dip, hand, sweep, kick nets and screens are rapid devices for collecting macroinvertebrates in wadable streams and rivers or at low tide in the inter-tidal zone of tidal sites. Two approaches are generally used, one in which the investigator sweeps the dip or hand net through aquatic habitats (Slack, _et al_., 1976; Armitage, _et al_., 1981) and one in which the kick net or hand held screen is held stationary against the streambed, facing upstream, and the investigator physically disturbs the stream bottom just upstream from the net or screen. The investigator vigorously kicks with the feet four or five times into the streambed to disturb the habitat in an upstream direction (Hynes, 1961; Morgan and Egglishaw, 1965; Frost, _et al_., 1971; Armitage, _et al_., 1974; Armitage, 1978; Hornig and Pollard, 1978; Pollard, 1981; and Plafkin, _et al_., 1989). The kicks disturb the substrate, dislodging the macroinvertebrates and some detritus, and cause the benthos to be swept by the current into the net. The debris and organisms in the kick net are then washed down into a sieve bucket and larger leaves and debris are removed.

5.15.3 Dredges are devices that are usually pulled by hand or power boat across or through the bottom sediment of a lake or stream to sample the benthos and prevent loss of active macroinvertebrates. The forward motion of the dredge carries macroinvertebrates into the net.

5.15.3.1 Elliott and Drake (1981a,b) compared four light-weight dredges for sampling in rivers. They indicated that the dredges are not suitable for quantitative sampling. Also, considerable variation existed in their effectiveness as qualitative samplers for estimating the total number of taxa per sample.

5.15.3.2 Dredges should be emptied after collection into a shallow tray, bucket, or sieving device if the sample is sorted on-site. The sample can be placed directly in labeled wide-mouth containers with preservative and transported back to the lab for processing.

5.16 Suction Samplers

5.16.1 Suction samplers have been used widely in sampling macroinvertebrates in fresh, estuarine, and marine waters (Brett, 1964; Larsen, 1974; Gale and Thompson, 1975). They can be placed directly on the sampling station and can be operated by hand in shallow water or by a scuba diver in deep water (see 5.18).

5.17 Photography

5.17.1 The use of photography is mainly limited to environments that have suitably clear water and are inhabited by sessile animals and rooted plants. Many estuarine habitats, such as those containing corals, sponges, and attached algal forms, fall in this category and can be photographed before, during and after the introduction of stress. The technique has been used with success in south Florida to evaluate changes brought about by the introduction of heated effluents.

5.17.2 The technique for horizontal underwater photos using scuba gear involves placing a photographically identifiable 1.0 m^2 area frame or marker in the habitat to be photographed and an additional nearby marker on which the camera is placed each time a photograph is taken. By this means, identical areas can be photographed repeatedly over a period of time to evaluate on-site changes in sessile forms at both affected and control stations. Vertical, overhead photos may be taken under suitable conditions.

5.17.3 Photographs are also useful in documenting a habitat or alterations in a station over time (e.g., increase in canopy cover, changes in channelization of a stream, and effects of flooding, etc.).

5.18 **Scuba**

5.18.1 This equipment can be used in freshwater sampling of mollusks in large riverine systems or with diver collected cores.

5.18.2 The reader is referred to Simmons (1977), Sommers (1972), U.S. Department of the Navy, U.S. Navy diving manual (latest edition), and Gale and Thompson (1974) for much additional information on this subject. All USEPA diving operations should be conducted in accordance with standards set forth in the U.S. EPA Occupational Health and Safety Manual-1440, 1986, entitled Chapter 10, EPA Diving Safety Policy. Therefore, if the need for diving capability exists, approval must be obtained through an USEPA regional laboratory diving officer. Scuba gear can be used to improve aquatic sampling; in particular sampling of mussels, other benthos, and fish. Isom, et al., (1979) reported utilizing scuba in rediscovery of snails, which were thought to be extinct. Various investigators had sampled the same areas previously on numerous occasions.

5.18.3 Gale (1977) notes the numerous applications of scuba to sampling benthos including placement and retrieval of artificial substrate; use of suction samplers (Larsen, 1974; Gale and Thompson, 1975); sampling with a quadrate frame; and, perhaps most importantly, identifying and delineating substrate types for purpose of determining sampling effort (stratified sampling) and choice of samplers.

5.18.4 If pelecypods (freshwater mussels) are to be sampled with brails in areas which historically contained them and/or it is desired to sample quantitatively, scuba can be used effectively in taking quadrates. In large rivers, which have mussel beds with homogenous substrate, it is desirable to take at least 10 square meter quadrates (10,000 square cm each). In small rivers where the mussels' niche may be between rocks and it is generally difficult to place a square meter frame, then a 0.5 square meter frame (2500 square cm) should be utilized with no less than 3 square meters, or twelve 0.5 square meter samples taken. Samples should be taken randomly in all cases, which in the latter instance, will result in collection of good representative diversity (see Section 2, Quality Assurance and Quality Control).

5.18.5 Scuba diving is safe if conducted by rigid safety standards, some of which are mandatory for scientific/educational diving (See Federal Register, July 22, 1977; 42, 41: pp. 37650-37673). Conformance with these and subsequent standards is costly but essential for safe conduct of scuba sampling. See references listed above for more in depth discussion of safety, the buddy system, etc. The need for observance of safety rules cannot be overemphasized.

5.19 Brails

5.19.1 This device is primarily limited to sampling of bivalve mussels in large (non-wadable) rivers.

5.19.2 The use of brails for commercial harvest of mussels has been the common practice since before 1900; however, this practice and scuba have been used by investigators to study mussel populations on a limited basis.

5.19.3 The reader is referred to Coker (1919), Van der Schalie (1941), Scruggs (1960), Lopinot (1967), Isom (1969), Bates (1970), Starrett (1971), and Buchanan (1980) for more information on collecting mussels, brails, and brailing. Coker (1919) describes how to make a brail.

5.19.5 Once the site to be sampled has been identified, reference should be made to historical literature for determination of species that may be encountered.

5.19.6 Quantitative sampling is accomplished with a crowfoot brail to determine the rate of catch per drag from a given area. All equipment can be made or rented from and fished by a commercial fisherman. Each brail sample consists of dragging a measured distance of 100 m, then sorting and counting the catch. The area sampled is calculated in square yards by multiplying the length of brail by 100 m. Catch success is expressed in terms of the average catch of mussels per square per drag. Brail sampling is randomized within fishing area and by time periods during two complete harvest seasons (March through August).

5.19.7 Brailing is also an effective qualitative sampling device, especially in large, deep rivers. Where possible, the services of a commercial mussel fisherman should be utilized. The experienced mussel fisherman is adept at using brails and only extensive experience would make an investigator's results equivalent to the general mussel fisherman. Maximum legal brail length is 16 feet (approximately 5 m) in some states; diameter of wire used for hooks is also controlled. These points can be worked out with the state permitting agency.

5.19.8 A minimum of six 100 m long hauls (drags) should be accomplished where a single brail is used. Most commercial fisherman use two brails simultaneously; thus, only three hauls would be required. Record the time for each haul; however, take about 20 minutes to make each haul since a very slow speed is best for catching mussels. If the hauls are made too fast, the catch will be small. If a significant mussel population is found, then qualitative or quantitative scuba (see 3.18, Scuba) samples should be taken. A minimum of 10 m^2 samples should be taken by scuba at each station. All specimens should be identified to species, growth cessation rings counted, and measured for determination of population age structure.

5.19.9 Mussel fishing with brails is highly dependent on experience of the user; however, they are very efficient in the hands of experienced users as attested to by almost 100 years of continuous use.

5.19.10 Availability of brailing equipment may be a deterrent to its use; however, if the method is adopted more widely by the scientific community, suppliers may develop to meet the need.

5.20 Other Mussel Collecting Methods

5.20.1 Mussels found in small or medium sized streams and rivers that can be waded are often found most numerous on bars where the pools break off into shoals. Sometimes, there are constrictions in streams at these points where weed beds can be found. Sample into the lower end of pools, around the weed beds, and in riffles/runs and fast-flowing water. A long-handled rake modified with a rectangular collection basket of one-quarter inch wire mesh, dredge dip net, or using the hands are the best method for sampling mussels from these habitats (Starrett, 1971). It is advisable to wear gloves and place a net below the area being sampled to catch small mussels that might otherwise not be collected.

5.20.2 Other collection techniques and procedures can be found in the 1941 Annual Report of the American Malacological Union. Information on collecting snails can be found in the same publication.

5.20.3 If rare or endangered species are collected, they should be returned to their habitat since it is illegal to take such species.

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SECTION 6

SAMPLE PROCESSING

6.1 Sieving

6.1.1 Samples collected with grabs, coring devices, and artificial substrates contain varying amounts of finely divided materials such as decomposed organic material, silts, clays, and fine sand. To reduce sample volume and expedite sample processing in the laboratory, these fines should be removed in the field by passing the sample through a U.S. Standard No. 30 sieve. Sieves may be commercial models or homemade sieves framed with wood or metal. Floating sieves with wooden frames reduce the danger of accidental loss of both sieve and sample when working over the side of a boat in deep waters. A sieve should contain no cracks or crevices in which small organisms can become lodged.

6.1.2 Sampling efficiency is increased by using sieves with smaller mesh openings (Mason et al., 1975; Barber and Kevern, 1974; and Zelt and Clifford, 1972). However, use of the smaller mesh size does not have an appreciable effect on the eutrophic classification based on common biotic indices. Precision based on coefficient of variation (CV) increased with smaller mesh size (Mason et al., 1975). Usually the increased length of time required to use the smaller mesh sieve sizes is not compensated for by the increased accuracy of results (Hummon, 1981). Also, organisms passing through the U.S. Standard No. 30 sieve are not macroinvertebrates by definition. (See Section 1, Introduction).

6.1.3 If at all possible, sieving should be done in the field immediately after the sample is collected and the captured organisms are still alive, but time can often be saved by returning to the laboratory with the samples unsieved and doing the sieving with a mechanical device such as the elutriation apparatus described by Worswick and Barbour (1974). If the sample is likely to include tubificid worms, leeches, or Turbellaria, a few representative specimens of each should be picked out before sieving and fixed in 10% buffered formalin or transported live to the laboratory for fixing or immediate identification. Once preserved, many organisms become quite fragile and if subjected to sieving will be broken up, lost, or rendered unidentifiable. Great care should be taken in sieving preserved samples containing mayflies, stoneflies and worms to reduce breaking the specimens or otherwise damaging body parts necessary for identification.

6.1.4 Sieving may be accomplished by one of several techniques depending upon the preference of the biologist. In one method, the sample is placed directly into a sieve and the sieve is then partially submerged in water and agitated until all fine materials have passed through. The sieve is agitated, preferably in a large tub of water but sieving may be done over the side of the boat if care is taken not to spill the sample. A variation of this technique is to place the original sample in a tub or bucket, add screened water, stir, and pour the resulting slurry through a U.S. Standard No. 30 sieve. Only a moderate amount of agitation is required to completely clean the sample. Since this method requires considerably less effort, most biologists may prefer it. A sieve bucket (Fig.11) described by Hiltunen (1983) for use in the Great Lakes works well under most conditions and allows the sample to be sieved while the boat is under way to the next sampling site. The cycle sieve described by Mason (1976) works well in calm weather from a small boat but is cumbersome and impractical for use from large boats, bridges or other such structures. In all of the above methods, remove, carefully clean, and discard all the larger pieces of debris and rocks from the sample before stirring or agitating.

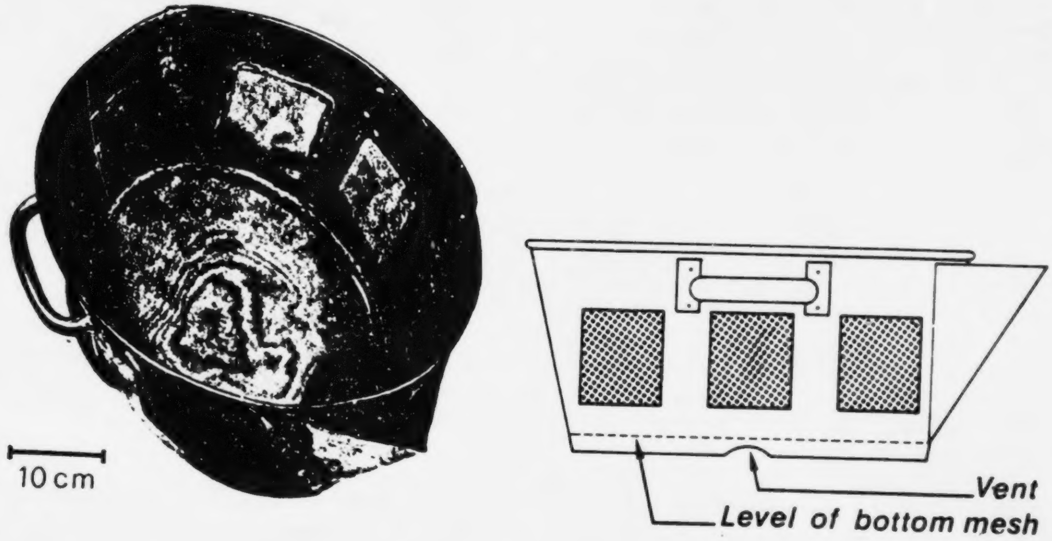

Figure 11. Great Lakes sieve bucket (From Hiltunen, 1983).

6.1.5 Artificial substrate samplers are placed intact into a bucket or tub of screened water and dismantled. Each individual piece of substrate is rinsed, gently but thoroughly cleaned under water with a soft brush such as a soft bristled toothbrush, examined visually, and laid aside. The water in the bucket or tub is then poured through a U.S. Standard No. 30 sieve to remove the fines. After most of the fines are washed from the sample, the organisms are left scattered over the surface of the screen. These organisms can be picked from the screen with forceps and placed in the sample container. A faster method is to concentrate them at one edge of the sieve by gently swirling the sieve in a little water, then tilting the sieve over a wide-mouth jar and gently backflush the organisms into the jar with water from a wash bottle directed through the screen.

6.1.6 Another way to separate the organisms from the detritus is the flotation method in which a concentrated aqueous solution of sugar, salt, or other chemical is poured over the sample in the tub or bucket causing the animals to float up out of the detritus due to the difference in the specific gravity of the animals and solution. The organisms can then be poured or scooped into the sample container with a sieve spoon. Some organisms, such as clams and snails, must still be hand picked from the debris because they are too heavy to float. Two or three lbs. of sugar per gallon of water makes a good flotation solution (Anderson, 1959).

6.1.7 When drift net or Surber-type samplers are being used, it is usually possible to empty the bag directly into a white bottom enamel pan or small bucket and hand pick the organisms into a sample container filled three-fourths full of preservative.

6.1.8 Although the U.S. Standard No. 30 (600 μm) sieve is also commonly used in marine studies, some investigators (Grassle *et al*. 1985) have chosen to use a 300 μm sieve in order to more efficiently sample smaller and juvenile macrofauna. This practice requires more time and taxonomic expertise. The 600 μm sieve is usually adequate since the vast majority of macrofaunal biomass and production is associated with larger forms.

6.1.8.1 For marine work the use of more than one sieve in series, one on top of the other, allows benthic communities to be fractionated by size allowing comparisons of community size distributions between stations and over time. Commonly used sieve sizes are 300 μm, 500 μm, 600 μm, 1 mm, and 2 mm.

6.1.8.2 Sieving marine samples should be done by rinsing organisms with a gentle spray of water to minimize mechanical damage to the organisms. Direct heavy jets of water should not be used and an elutriate procedure that ensures that the major source of water is from the bottom of the sieves is recommended. Water used in sieving should be obtained from the sample site whenever possible. Fresh water should never be used to sieve unpreserved marine fauna because of osmotic effects that cause cell bursting.

6.2 Preservation and Fixation

6.2.1 All samples collected in the field should be preserved in 70-80% ethyl alcohol (ethanol), but ideally, and for ease in identification, representative specimens of leeches, aquatic oligochaetes, and other soft bodied organisms, if time permits, should first be fixed in 10% formalin to fix the tissue. After fixation (about 10 minutes), depending on size and number of organisms, or after returning to the laboratory, they may be preserved in 70-80% ethanol. This process should aid in their identification (see Section 6.5.4. and 6.5.5). Because wash water is contained in the sieved material, the stock preservative solution added to the sample should be over-strength (90%) so that the final solution will be sufficient to preserve the organisms. Grab samples collected from lakes, the muddy bottoms of large rivers, estuaries and oceans are often fixed and preserved in ten percent buffered formalin because they contain many worms which are difficult to identify after being preserved in ethanol. Formalin should be buffered to a neutral or slightly alkaline level with borax.

6.2.2 Since leeches dropped alive into preservatives such as 70-80% ethanol or 10% formalin solution contract strongly, some diagnostic features used for species identification may be difficult to determine by the inexperience. Ideally, specimens should first be narcotized by direct placement into carbonated water, fixed in 10% formalin, and preserved in 70-80% ethanol. If this procedure is inconvenient in the field, the specimens should be preserved directly in 70-80% ethanol. Most specimens still can be identified to species but might take a little longer than usual. Additional collecting, narcotizing, and processing techniques can be found in Klemm (1982, 1985).

6.2.3 Turbellarians that require identification to species should be transported to the laboratory alive in a small amount of water (Pennak, 1978, 1989).

6.2.4 Although not always necessary, species identifications are easier and morphometric analyses are facilitated if marine organisms are relaxed after sieving and prior to fixation and preservation. Organisms to be relaxed are transferred from sieves to a fine mesh (approximately 100 µm) bag and placed in a solution of magnesium chloride (approximately 75 g/l) for about 10 minutes. The organisms may then be fixed and preserved.

6.2.4.1 A 10% (by weight) formalin solution is most commonly used to fix and preserve marine samples. The solution is buffered to keep the dissolution of molluskan shells to a minimum.

6.2.4.2 Because formaldehyde is a carcinogen, and because some individuals develop severe sensitivities to formaldehyde over time, some researchers prefer to transfer samples from formalin to ethanol for preservation. This is acceptable if samples are only to be used to do taxonomic studies. However, biomass measurements should not be done on samples preserved in ethanol. Although weight loss due to preservation in formalin is significant (10-20%) (Mills et al., 1982; Schram et al., 1981; Williams and Robins 1982), weight loss due to preservation in ethanol is greater.

6.2.5 Sample containers used for holding preserved samples should be large enough so that they are not over one-half full of the washed sample before the preservative is added. Quart or liter sized jars are adequate for most samples collected with artificial substrate, drift net, or square-foot type samplers, but two or more jars may be needed for a grab sample depending on the amount of detrital material mixed with the sample. Hand picked specimens are usually preserved by placing them directly into small screw-cap vials filled with 70-80% ethanol.

6.2.6 If the samples are not sorted within two or three weeks after collecting, the preservative should be poured off and replaced with fresh preservative for permanent storage (Cairns and Dickson, 1971).

6.2.7 After sorting and/or identification most macroinvertebrates should be stored in a solution of 70-80% ethanol and 5% glycerine in vials sealed with tightly fitting rubber stoppers. If screw-cap vials are used, they should be submerged in 70-80% ethanol in a larger container and should be checked yearly to replace alcohol lost because of evaporation or Teflon tape can be used to secure the screw-caps to prevent evaporation.

6.3 Labelling and Record Keeping

6.3.1 All sample containers must be labeled in the field immediately upon collection. Sample labels made of water-resistant paper should be placed inside each sample container. Write all information on the label with a soft-lead pencil or waterproof ink. Where the volume of sample is so great that several containers are needed, additional external labels with sample number and notations such as 1 of 2, 2 of 2, etc. are helpful for identifying the sample containers when the samples are logged in at the laboratory. All labels must include a sample identification number which corresponds to the number entered in the field notebook for that sample, the sampling date, water body and location from which the sample was collected, and the name of the collector. In addition to the information on the label, the field notebook should include the sampling method, weather, substrate characteristics, depth, and any other physical or environmental conditions noted.

6.3.2 Marine sample data sheets should include date of collection, time of day, station number, geographic coordinates, replicate number, core penetration depth, and the identification number and final storage location of each sample. These data sheets should also include space for comments on the visual appearance of each sample (e.g., obvious tubes or burrows, presence or absence of a surface flocculent layer, sediment color, apparent depth of the redox-potential discontinuity, etc.); ancillary data such as water temperature, salinity, secchi disk visibility, vertical profiles of dissolved oxygen; and other data potentially useful in the interpretations of benthic community data.

6.3.3 As soon as possible after returning to the laboratory, each sample should be assigned an ID number in sequence. This number identifies the sample in a bound ledger where all the information from the field label and field notebook are recorded for permanent record. The sample ID number must also be placed prominently on the sample container before storing so that it can be identified when needed. This sample ID number should be placed on all specimen vials, microscope slides, and other items connected with the sample.

6.4 Sorting and Subsampling

6.4.1 Sorting

6.4.1.1 Sort through the samples by hand in the laboratory using a low power (2X) scanning lens or a stereomicroscope. Place one or two tablespoonfuls of the sample in a white enamel pan (size 25 X 40 X 5 cm) filled about one-third full of water. Usually small insects and worms will float free of most of the debris when ethanol-preserved samples are transferred to the pan. These floating organisms should be removed before they soak up water and sink. They can be skimmed off with a sieve spoon or poured off. Addition of about one tablespoon full of sugar and stirring the sample will cause most of the other organisms to float free. Flotation in formalin-preserved samples is accomplished by adding sugar slowly to raise the specific gravity to 1.12 (Pask and Costa, 1971). Numerous other techniques have been proposed to aid recovery of the organisms from the sample debris, including solutions of magnesium sulphate, D-mannitol, calcium chloride or sodium chloride; electricity; bubbling air through samples in a tube, etc. The efficacy of these techniques is affected both by the characteristics of the substrate material and the types of organisms present (Flannagan, 1973). Regardless of the sorting method used, heavy organisms such as clams and snails will not float and will have to be picked out with forceps.

6.4.1.2 Various staining methods have been devised to help speed the sorting process (Williams and Williams, 1974). Staining samples in the field with either rose bengal or phloxine B at a concentration of 100 g/L of ethanol or formalin significantly reduces sorting time for benthic samples (Mason and Yevich, 1967). Should the stain interfere with identifications where color patterns or internal organs must be examined, the stain can be removed by placing the organisms in 95% ethanol over night.

6.4.1.3 As soon as the sample is sorted, make note in the log book, including the date and the initials of the person who sorted the sample. It is often advisable to ask a co-worker to check the sample debris before discarding to be certain no organisms were overlooked. The organisms may be sorted and transferred to watch glasses or petri dishes for immediate identification and counting, or stored in vials for future identification.

6.4.2 Subsampling

6.4.2.1 Analysis time for samples containing large numbers of organisms can be substantially reduced if the samples are subdivided before sorting. There are several methods for subdividing the samples and each method has its advantages and disadvantages.

6.4.2.2 Welch (1948) described a method that has been used successfully for many years. The sample is thoroughly mixed and distributed evenly over the bottom of a shallow white-bottom pan. A divider, delineating one-quarter sections, is placed in the tray and one quarter or two opposite quarters are sorted.

6.4.2.3 An air driven subsampler (Figure 12) was described by Wrona et al. (1982) and modified by the State of Maine Department of Environmental Protection (Susan Davies, Personal communication). The sample is placed in a Imhoff-type settling cone that is filled with water to a total volume of one liter. The sample is gently agitated for two to five minutes by use of an air stone sealed into the bottom and connected to an air supply. One-quarter of the sample is removed with a wide-mouth 50 mL dipper or test tube in five aliquots and combined in a white-bottom pan for hand sorting. If less than 100 organisms are present in the one-quarter subsample, additional one-quarter subsamples are removed until the subsample contains at least 100 organisms. Large or heavy organisms that cannot be suspended by agitating the water are sorted and counted separately.

6.4.2.4 The Rapid Bioassessment Protocols II and III (Plafkin et al., 1989) use a modification of a subsampling method described by Hilsenhoff (1987).

All large detrital material (leaves, twigs, etc.) are rinsed, visually inspected for organisms, and discarded. The sample is then poured into a white-bottom pan that has been marked with a grid pattern of 5-cm squares. Grids are randomly selected and all the organisms in the selected grids are picked in succession until approximately 100 organisms have been removed from the sample. All the organisms in the grid that contains the 100th organism are picked once that grid is started. Before using this method, live organisms should be narcotized with club soda or nicotine before sorting so they will not move from square to square.

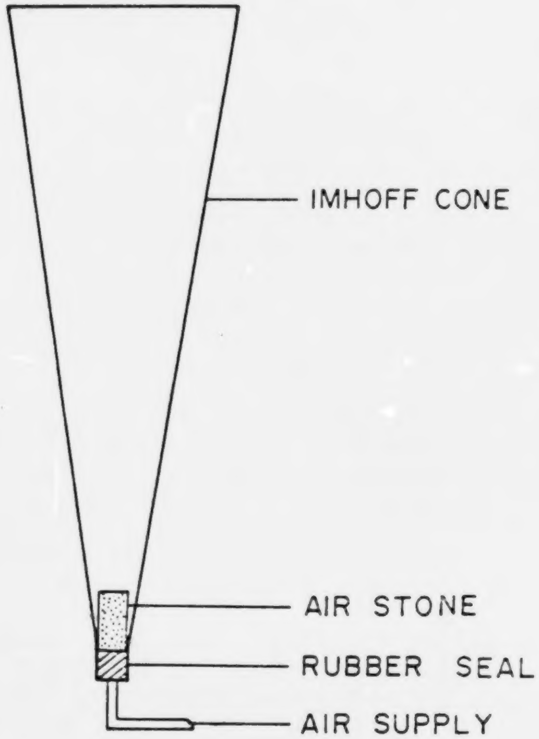

Figure 12. Imhoff cone subsampler (From Wrona et al., 1982).

6.4.2.5 Regardless of the method used for subsampling, the sorted sample should be labelled to reflect the portion sorted (e.g., 2X if half sorted, 4X if one-quarter sorted, 100 C if 100 count method was used, etc.) with the sample ID number. The unsorted portions of the sample should be combined, preserved, labeled and stored for future reference. It should be discarded only if there is no possible future need.

6.4.2.6 Experience has shown that, if less than one-quarter of the original sample is sorted, considerable error may result in estimating the total numbers of worms and other organisms that tend to clump. If the sample contains large numbers of a single taxonomic group (such as oligochaete worms or midges) but few other organisms, it may be advisable to subsample the abundant taxa and pick all of the other organisms.

6.5 Preparation of Microscope Slide Mounts

6.5.1 To identify certain taxa of macroinvertebrates, it is often necessary to make slide mounts of all or parts of the organisms for examination under a compound microscope. Generally, if the organism is over 10 mm in length, it is best to carefully remove the important diagnostic structures (such as mouthparts or genitalia) with fine pointed forceps and mount them on microscope slides. Some large chironomids and tubificid worms that are too long to be mounted whole are cut in half and mounted under two separate cover glasses on the same slide.

6.5.2 Because most of the slides made for diagnostic purposes will be discarded after the organisms have been identified, we recommend mounting directly from the preservative using a water miscible mounting medium consisting of a mixture of two-thirds CMCP-9 and one-third CMCP-9AF (Beckett and Lewis, 1982). This mixture stains the organism a light red and contains a clearing agent providing optimum contrast for easy viewing of taxonomically important structures after about 12 hours clearing time. Because CMCP-9/9AF is a low viscosity medium, the specimen can be easily manipulated after the cover glass is in place by using pressure from forceps on the cover glass, rolling the specimen while viewing with a dissecting microscope until the best viewing position is obtained. The slides may be made permanent by ringing the cover glass with additional CMCP-9/9AF followed 24 hours later with polyurethane spar varnish or fingernail polish. Round 12 mm or 15mm cover glasses are recommended because they are less likely to trap air bubbles, are easier to manipulate, and less likely to break with pressure than the square ones. This method has proven very successful for making semi-permanent slides of whole chironomids and oligochaetes and parts of mayflies, caddisflies, and other macroinvertebrates.

6.5.3 Other slide-making techniques have been recommended for specific groups of organisms (Mason, 1973; Beck, 1975; Britton and Greeson, 1988). Although these methods are more time consuming and require more effort than the above method, they are thought to produce superior results by some taxonomists and are considered more permanent.

6.5.3.1 Many chironomid taxonomists use KOH to clear the midges before mounting them in Euparal (Mason, 1973) or CMCP-10 (Beck, 1975). The US Geological Survey (Britton and Greeson, 1988) has adopted a slightly modified version of this method for mounting midges and blackflies as follows:

1. Place the specimens in distilled water for 10 minutes to remove the preservative.
2. Transfer to crucibles containing 10% KOH and heat for 10 to 15 minutes to digest opaque tissue, taking care not to digest exoskeleton also.
3. Soak in distilled water for at least 3 minutes to remove KOH.
4. Soak in 95% ethyl alcohol for three to five minutes.
5. Mount in a drop of Euparal or CMCP-10.
6. Place specimen ventral side up and cover with a 12 mm cover glass.

7. Working under a stereoscopic microscope, apply pressure from a pencil eraser to roll ventral side up and flatten the head capsule.
8. Allow the slide to dry for about a week before storing on edge.

6.5.3.2 Water mites mounted using either of the above methods are nearly impossible to identify beyond family level. If identification to genus or species is needed, the mites should be dissected first to speed the clearing process and make it possible to examine sclerotized plates and other structures on both dorsal and ventral surfaces of the abdomen. First, using a dissecting microscope, forceps and a needle, separate one palp or the entire gnathostoma with palps from the body and mount the palps in the position shown in Figure 13. Next, separate the dorsum of the abdomen from the venter leaving a small section of the posterior body wall intact as shown in Figure 14, and mount with the venter and dorsum upward. Rather than dissect the very small specimens, pierce the body wall in the posterior-lateral areas to facilitate the clearing process and mount with the ventral surface upward (Britton and Greeson, 1988).

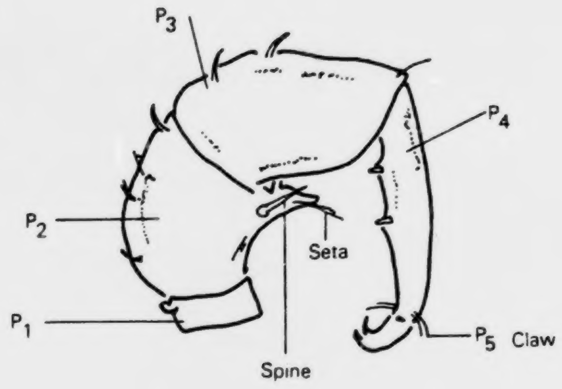

Figure 13. Five-segmented palp of a water mite (From Britton and Greeson, 1988).

6.5.3.3 When permanent slides are needed for the water mites, the double cover-glass glycerine method described by Mitchell and Cook (1952), modified by Britton and Greeson (1988), and illustrated in Figure 15 should be used.

6.5.4 Aquatic oligochaete worms--To identify oligochaete worms the specimens must be go through a clearing process and be side mounted. The identification of species requires a compound light microscope and some specimens require oil immersion (1000X). Some worm specialists make temporary mounts by placing oligochaete specimens on sides in Amman's lactophenol (100 g phenol, 100 ml lactic acid, 200 ml glycerine, 100 ml water), a medium which clears tissues and eliminates the risk of specimen desiccation if a more permanent

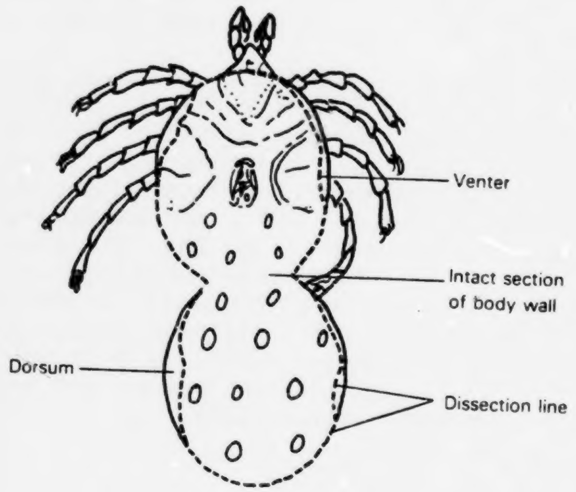
Venter
Intact section of body wall
Dorsum
Dissection line

Figure 14. A water mite showing the dorsum separated from the venter, leaving a small section of the posterior body wall intact (From Britton and Greeson, 1988).

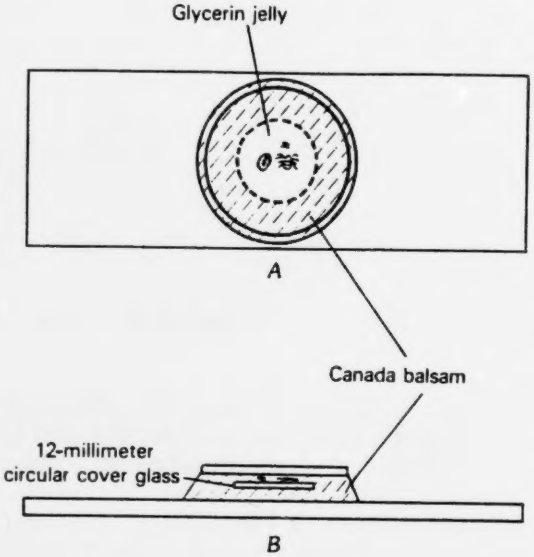
Glycerin jelly
A
Canada balsam
12-millimeter circular cover glass
B

Figure 15. Top (A) and side (B) views of the double cover-glass technique for mounting aquatic water mites (From Britton and Greeson, 1988).

mount cannot be prepared immediately following extraction from the sample (Brinkhurst, 1986; Hiltunen and Klemm, 1980, Stimpson, et al., 1982; or Klemm, 1985). The clearing process usually takes a few hours to a few days depending on the size of the specimens. Gentle application of heat will speed the clearing process. If the specimens are preserved in 70-80% ethyl alcohol, they should be placed in 30% ethyl alcohol and then in water for a short time to leach out the alcohol before clearing. The alcohol retards the clearing process of Amman's lactophenol (Hiltunen and Klemm, 1980, Stimpson et al., 1982; Klemm, 1985). Do not leave specimens in the water too long (not more than two hours) because the worms will begin to deteriorate. Naidids and tubificids can be held indefinitely in Amman's lactophenol or 10% buffered formalin for later processing and mounting.

6.5.4.1 Non-resinous media are recommended for rapid processing of large numbers of specimens. For extremely important reference specimens, a permanent resinous mounting medium is best.

6.5.4.2 The non-resinous semi-permanent mounting media (CMCP-9 or 9AF, CMCP-10, or aquamount), which also contain clearing agents, are the simplest to use, allow for rapid processing of specimens, and are usually adequate for species identification. If Fuschin dye is added to the colorless mounting media (CMCP-9 or CMCP-10), only enough of the dye should be used (avoid overstaining) to slightly or partially stain the specimens. The specimens can be mounted directly on the slide using these media. However, the clearing process of these media takes approximately 24 hours. If the slides are to be semi-permanent, the edge of the cover slip should be sealed with finger nail lacquer to prevent the mounting medium from shrinking and forming bubbles under the cover slip. An 18 mm diameter, No. 0 or 1 round cover glass is appropriate because it will adequately accommodate the size range of the worms and the shape allows for maneuvering the specimen to rest in the most desired position by gentle rotation of the cover glass.

6.5.4.3 Place naidids or tubificids on their sides so that both dorsal and ventral fascicles of chaetae can be examined (Hiltunen and Klemm, 1980; Stimpson et al., 1982; Klemm, 1985). A variation from this is followed with specimens of Dero which must be viewed from the dorsal aspect, revealing the arrangement of the branchial apparatus (Hiltunen and Klemm, 1980, Klemm, 1985). The methods sections found in Hiltunen and Klemm (1980)and Klemm (1985) should be consulted for more specific information on identification of specimens.

6.5.4.4 Optimal resolution and longevity of mounted materials are achieved only in resinous media (e.g., Canada Balsam, Harleco's Xylene Coverbond, etc.). These mounting media require dehydration of the specimens through the alcohol series and clearing before mounting in Canada balsam or other resinous medium, but they produce the best permanent mounts (Knudsen, 1966; Klemm, 1985).

6.5.5 Leeches--species identification of most specimens do not require mounting on slides. A stereozoom microscope of 500X is needed for species identification. However, specialized slide-making techniques must be used for species identification of some leeches (See Klemm, 1982, 1985, 1990).

6.5.6 Regardless of the mounting method used or the permanence of the slides, proper labelling is a must. The label should include the date the slide was made, the sample ID number, and the initials of the person who made the slide. Labels on permanent slides should also include the location of the collecting site and name of the collector.

6.6 Drying Methods

6.6.1 Occasionally, alcohol-preserved specimens may require dry mounting on points or minutens for identification. The critical point drying method is recommended because the pigments colors are preserved, specimens do not collapse, and they are not brittle. Specimens to be dried are taken from 80% ethanol and passed through the alcohol series of washes in a small mesh screen basket with a lid, ending with two washes in 100% ethanol. After removal from the alcohol wash, the specimens, with the basket, are placed in the chamber of the critical point drier and processed according to dryer instructions (Gordh and Hall, 1979).

6.7 Organism Identification

6.7.1 The taxonomic level to which animals are identified depends on the needs, experience, and available resources. However, species level identification is very important in determining water quality and environmental pollution (Resh and Unzicker, 1975). The rapid bioassessment protocol II calls for organism identification only to the family level for use with Hilsenhoff's (1988) Family Biotic Index, whereas protocol III calls for identification to genus or species if possible (Plafkin et al., 1989). Many state programs carry most organism identifications to the genus level, while others (e.g., State of Maine) carry identification of certain taxa, such as stoneflies and mayflies, to species. Although the selective sensitivity of a family-level identification effort is often sufficient for differentiating non-impaired, moderately impaired, and severely impaired conditions, subtle differences in biological impairment will not be discerned except by species-level identification (Plafkin et al., 1989). In general, identifications should be carried to the lowest taxonomic level readily possible, and the taxonomic level to which identifications are carried in each major group should be constant throughout a given study.

6.7.1.1 Since the accuracy of identification depends on the availability of up-to-date taxonomic literature. A library of the basic taxonomic literature is essential for benthic laboratories. Basic references that should be available in a macroinvertebrate identification laboratory are listed in Section 8, Taxonomic Bibliography.

6.7.2 For comparative purposes and quality control checks, a reference collection of identified specimens should be established in each laboratory.

6.7.3 Most identifications to order and family can be made using a hand lens or a stereoscopic microscope with up to 50X magnification. Identification to genus and species often requires a compound microscope with phase contrast capable of 1000X magnification. Preparation of specimens for microscopic viewing is discussed in Section 6.5.

6.7.4 Insect larvae often comprise the majority of macroinvertebrates collected with artificial substrate samplers, drift nets, and other net type devices. In certain cases, identifications are facilitated if exuviae, pupae, and adults are available.

6.7.5 The life history stages of an insect can be positively associated only if specimens are reared individually. Small insect larvae can be reared individually in 6 to 12 dram vials half filled with stream water and aerated by use of a fine-drawn glass tubing. Mass rearing can be carried out by placing rocks and sticks containing the larvae in an aerated aquarium. Current can be provided in the aquarium by use of a magnetic stirrer (Mason and Lewis, 1970).

6.7.6 As organisms are identified, the individuals in each taxonomic category are counted and the numbers recorded on bench sheets (see Appendix C). Samples are compared by use of a summary sheet (see Appendix D) which provides room for comparing eight samples from the same sampling site.

6.8 Biomass

6.8.1 Macroinvertebrate biomass (weight of organisms per unit area) is a useful quantitative estimation of standing crop and is useful in assessing the biological integrity of surface waters. One study shows that biological assessments of water quality status using biomass estimates of wet, dry, and ash-free dry weights provide essentially similar results concerning impact of a sewage treatment plant discharge as did counts of individual organisms using a variety of commonly utilized biotic indices of water quality (Mason et al., 1983, 1985). To determine wet weights, soak the organisms in distilled or deionized water for 30 minutes, centrifuge for one minute at 140 g in wire mesh cones, and weigh to the nearest 0.1 mg. To obtain dry weight, dry the organisms to a constant weight at 105 degrees C for 4 hours or vacuum dry at 105 degrees C for 15 to 30 minutes at one-half atmosphere. Cool to room temperature for 15 minutes and weigh to nearest 0.1 mg. Freeze drying (-55 degrees C, 10 to 30 microns pressure) can be used. It has advantages over oven drying because the organisms remain intact for identification and reference, preservatives are not needed, and cooling the material in desiccators after drying is not required. The main disadvantage of freeze drying is the time (usually 24 hours) required for drying to a constant weight. To obtain ash-free dry weight, ash the dried organisms at 500 degrees C for one hour. Cool the ash to ambient temperature in a desiccator and weigh to the nearest 0.1 mg. Express the biomass as ash-free dry weight.

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SECTION 7

DATA EVALUATION

7.1 Introduction

7.1.1 One of the major concerns of USEPA, other federal, state and private agencies is to describe water quality and habitat quality in terms which are easily understood by the non-biologist. The purpose of this section is not to recommend one particular data evaluation method, but to point out a number of more common methods. Some of these methods may not be applicable to every stream or water body in the United States.

7.1.2 Water quality and habitat quality are reflected in the species composition and diversity, population density and biomass, and physiological condition of indigenous communities of aquatic organisms. A number of data interpretation methods have been developed based on these community characteristics to indicate the water quality and the degree of habitat degradation, and also to simplify communication problems regarding management decisions.

7.2 Analyses of Qualitative Data

7.2.1 As previously defined, qualitative data result from samples collected in such a manner that no estimates of numerical abundance or biomass can be calculated. The principle output is a list of taxa collected in the various habitats of the environment studied. The numerous schemes advanced for the analysis of qualitative data may be grouped under two categories; the indicator organism scheme and reference station methods.

7.2.2 Indicator Organism Scheme

7.2.2.1 For this technique, individual taxa are classified on the basis of their tolerance or intolerance to various levels of domestic wastes (Beck, 1954; Lewis, 1974; Chutter, 1972; Hilsenhoff, 1977; Howmiller and Scott, 1977; Milbrink, 1983; Reynoldson *et al*. 1989). Taxa are classified as tolerant or intolerant according to their presence or absence in different environments as determined by field studies. Beck (1955), reduced data, based on the presence or absence of indicator organisms, to a simple numerical form for ease in presentation. Clean water taxa are given twice the weight as tolerant organisms in the formula:

$$2\ (n\ \text{Class I}) + (n\ \text{Class II}) = \text{Biotic Index}$$

where "n" is the number of taxa in that class. Values less than 10 are considered to indicate a polluted stream.

7.2.3 Reference Station Methods

7.2.3.1 Reference station methods (Ohio EPA, 1989) compare the characteristics of the fauna in clean water habitats with those of fauna in habitats subject to stress. Patrick (1950) compared stations on the basis of richness of species, and Wurtz (1955) used indicator organisms in comparing stations.

7.2.4 If adequate background data are available to an experienced investigator, both of these techniques can prove quite useful; particularly for demonstrating the effects of gross to moderate organic contamination on the macroinvertebrate community. To detect more subtle changes in the macroinvertebrate community, quantitative data on numbers or biomass of organisms are needed. Data on the presence of tolerant and intolerant taxa and richness of species may be effectively summarized for evaluation and presentation by means of line graphs, bar graphs, pie diagrams, histograms, or pictorial diagrams (Ingram and Bartsch, 1960).

7.2.5 Classification of representative macroinvertebrates according to their tolerance of organic wastes is presented in Appendix A. Hilsenhoff's (1977) original tolerance classification with a numerical range of 0 to 5 is followed in Appendix A. Later, Hilsenhoff (1987) modified his biotic index for Wisconsin taxa to include more intermediate values with a numerical ranged of 0-10. However, similar results can be obtained using index values of either 0-5 or 0-10, and adequate information is not available for many species that would allow use of the more definitive 0-10 tolerance range (Hilsenhoff, 1990, personal communication). In most cases, the taxonomic nomenclature used is that of the original authors listed at the end of Appendix A. The pollutional classifications were arbitrarily placed in three categories--tolerant, facultative, and intolerant--defined as follows:

- Tolerant: Organisms frequently associated with gross organic contamination, that are generally capable of thriving under anaerobic conditions. Tolerance values 4 and 5.
- Facultative: Organisms having a wide range of tolerance that frequently are associated with moderate levels of organic contamination. Tolerance values 2 and 3.
- Intolerant: Organisms that are usually not found associated with organic contaminants and are generally intolerant of even moderate reductions in dissolved oxygen. Tolerance values 0 and 1.

When evaluating qualitative data in terms of material such as that contained in Appendix A, the investigator should keep in mind the pitfalls mentioned earlier, as well as the following:

7.2.5.1 Since tolerant species may be found in both clean and degraded habitats, a simple record of their presence or absence is not of significance. However, the presence of intolerant organisms provides evidence of only one condition--clean water. But the fact that sensitive (intolerant) species may be totally absent, because of the discharge of toxic substances or thermal pollution, would indicate that absence of intolerant species may not be a reflection of the presence of organic wastes. The presence of tolerant organisms is a significant indicator of organic pollution only when they are dominant in the sample.

7.2.5.2 The presence or absence of particular taxa may depend more on characteristics of the environment, such as velocity and substrate, than on the level of degradation by organic wastes. This affects both the original placement of the taxa in the classificatory scheme and its presence in study samples.

7.2.5.3 Because indicator species evaluations are based on the presence or absence of organisms, a single specimen has as much weight as a large population. Therefore, studies may be biased by the drift of organisms into the study area. The technique is totally subjective and dependent upon the skill and experience of the individual who makes the field collections. Therefore, results of one investigator are difficult to compare with those of another, particularly where data are summarized in an index such as that proposed by Beck (1955).

7.2.6 Biotic Index

7.2.6.1 Many of the problems discussed above can be overcome by use of the biotic index proposed by Chutter (1972) and modified by Hilsenhoff (1977) for use with the index values given in Appendix A. Any organisms not listed in Appendix A should be given an index of three (3) unless available information would suggest a different value. This same formula is used with the family level biotic index of Hilsenhoff (1988a) and the Rapid Bioassessment metric 2 of Protocol III (Plafkin _et_ _al_., 1989) where pollution tolerance values of 0-10 are used. Appendix B gives the family level index values (Hilsenhoff, 1988a) for use with the family level biotic index. Results are comparable between stations in the same and nearby streams if similar habitats were sampled using similar methods and sampling effort (Hilsenhoff, 1988a,b). The formula to use is:

$$HBI = \Sigma \frac{n_i \; a_i}{N}$$

Where "n_i" is the number of individuals in the "i^{th}" taxa, "a_i" is the index value of that taxa, and "N" is the total number of individuals in the sample. Biotic index values below 1.75 indicate excellent water quality, 1.76-2.50 indicate good water quality, 2.51-3.75 indicate fair water quality, 3.76-4.00 indicate poor water quality, and over 4.00 would indicate serious water quality problems.

7.2.6.2 The following are water quality values for Hilsenhoff's (1988a) family level biotic index: 0.00-3.75 (excellent), 3.76-4.25 (very good), 4.25-5.00 (good), 5.01-5.75 (fair), 5.76-6.50 (fairly poor), 6.51-7.25 (poor), and 7.26-10.00 (very poor).

7.3 Analyses of Semi-quantitative and Quantitative Data

7.3.1 The high variability usually associated with benthic macroinvertebate populations makes them difficult to study quantitatively because of the large number of samples needed to obtain normal levels of precision. For most benthic studies, it is generally impractical, due to large number of samples needed, to detect population changes of less than 100% of the mean. Many benthic populations exhibit such high variability (see Section 4.5.) that any reasonable number of replicate samples would be too small to detect a population density difference of more than 200% of the mean between two sites (Schwenneker and Hellenthal, 1984). It is important to keep this limitation in mind as one considers the methods to use in evaluating the data.

7.3.2 Data from quantitative samples may be used to obtain total standing crop of individuals, or biomass, or both and numbers or biomass, or both, of individual taxa per unit area or unit volume or sample unit. Data from quantitative samples may also be evaluated in the same manner as discussed for qualitative samples but results will be qualitative. In order to reduce the amount of time spent in field sampling, there has been a recent trend to collect data based on level of effort or other not strictly quantitative methods and treat the data as semi-quantitative. These data are then analyzed using the quantitative methods described in this section.

7.3.3 For purposes of comparison and to provide data useful for determining production, a uniform convention must be established for the units of data reported. For this purpose, USEPA biologists should adhere to the following units:

- Data from devices sampling a unit area of bottom are reported in grams dry weight or ash-free dry weight per square meter (gm/m^2), or numbers of individuals per square meter, or both.

- Data from multiplate samplers are reported in terms of the total surface area of the plates, as grams dry weight or ash-free dry weight or numbers of individuals per square meter, or both.

- Data from rock-filled basket samplers are reported as grams dry weight, ash-free dry weight, or numbers of individuals per sampler, or both.

7.3.4 Three informative parameters of benthic community structure which may be obtained from quantitative grab or artificial substrate sample data are standing crop (biomass or numbers), species richness, and species composition. Standing crop and species richness in a community are highly sensitive to natural environmental conditions and to anthropogenic perturbations resulting from the introduction of contaminants. These parameters, particularly standing crop, may vary considerably in unpolluted habitats, where they may range from the typically high standing crop of littoral zones of glacial lakes to the sparse fauna of torrential soft-water streams. Thus, it is important that comparisons be made only between truly comparable habitats. Typical responses of standing crop or species richness to various types of stress are shown in Table 7 below:

7.3.5 Organic enrichment and sludge deposits are frequently associated. The responses shown are by no means simple or fixed and may vary depending on a number of factors including a combination of stresses acting together or in opposition, indirect effects (such as the destruction of highly productive vegetative substrate by temperature alterations, sludge deposits, turbidity, or chemical weed control) and the physical characteristics of the stressed environment; particularly in relation to substrate and current velocity.

Table 7. TYPICAL RESPONSES TO VARIOUS TYPES OF STRESS BY PARAMETERS OF BENTHIC COMMUNITY STRUCTURE

Stress	Standing crop (Numbers or Biomass)	Number of Taxa
Toxic substance	Reduces	Reduces
Severe temperature changes	Variable	Reduces
Silt	Reduces	Reduces
Low pH	Reduces	Reduces
Inorganic nutrients	Increases	Variable
Organic enrichment (Low DO)	Increases	Reduces
Sludge deposits (Non toxic)	Increases	Reduces

7.3.6 Data on standing crop and species richness may be presented in simple tabular form or pictorially with bar and line graphs, pie diagrams, and histograms. Whatever the method of presentation, the number of replicates and the sampling variability must be shown in the tables or graphs. Sampling variability may be shown as a range of values or as a calculated standard deviation, as discussed in Section 7.6.

7.3.7 Data on standing crop and species richness are amenable to simple but powerful statistical techniques of evaluation. Under grossly stressed situations, such analyses may be unnecessary; however, in some cases, the effects of environmental perturbations may be so subtle in comparison with sampling variation that statistical comparisons are a helpful and necessary tool for the evaluation process. For this purpose, biologists engaged in studies of macroinvertebrates should familiarize themselves with the simple statistical tools discussed in Section 7.6.

7.3.8 The usefulness of species composition as a parameter of environmental quality is based on the generally observed phenomenon that relatively undisturbed environments support communities having large numbers of species with no individual species present in overwhelming abundance. If the species found in a random sample from such a community are ranked on the basis of their numerical abundance, there will be relatively few species with large numbers of individuals and large numbers of species represented by only a few individuals. Many forms of stress alter species composition by making the environment unsuitable for some species or by giving other species a competitive advantage.

7.3.9 It is important for the investigator to keep in mind that there are naturally occurring severely stressed environments supporting communities dominated by one or more species adapted to rigorous conditions. Examples include the profundal fauna of deep lakes and the black fly dominated communities of the high gradient, bedrock section of a torrential stream. Furthermore, because colonization is by chance, both species richness and species composition may be highly variable in a successional community; for this reason, data summarized from artificial substrate samples must be evaluated with caution. These confounding factors can be reduced by comparing data from similar environments and by exposing artificial substrate samplers long enough for a relatively stable community to develop.

7.3.10 Data on species composition may be summarized and evaluated using percent species composition tables, frequency distribution tables and/or graphs; however, for any appreciable number of samples, such methods of presentation are so voluminous that they are virtually impossible to compare and interpret. Fortunately, single numerical values which provide a measure of species composition can be extracted from indices of diversity as proposed by Margalef (1957) and subsequently utilized by numerous workers (McIntosh, 1967; Cairns and Dickson, 1971; Wilhm and Dorris, 1968). Mean diversity ($\bar{d}$) may be calculated using the machine formula presented by Lloyd, Zar, and Karr (1968) and better known as the Shannon-Weaver mean diversity (Shannon and Weaver, 1963).

$$\bar{d}=\frac{C}{N}\left(N \log_{10} N - \Sigma n_i \log_{10} n_i\right)$$

where C=3.321928 (converts base 10 log to base 2); N = total number of individuals; and n_i = total number of individuals in the i^{th} species. When their table (see Table 23) is used, the calculations are simple and straightforward, as shown in Table 8.

Table 8 EXAMPLE OF CALCULATION OF MEAN DIVERSITY

	Taxa Number	Number of Individuals in each Taxon (n_i)	$n_i \log n_i$ (From Table 23)
	1	41	66.1241
	2	5	3.4949
	3	18	22.5949
	4	3	.4314
	5	1	.000
	6	22	29.5333
	7	1	.0000
	8	2	.6021
	9	12	12.9502
	10	4	2.4082
Totals	10	109	139.1391

N log N (109) = 222.0795 (From Table 23)

$\Sigma n_i \log_{10} n_i$ = 139.1391 (From Column 3 above)

$$\bar{d} = \frac{3.321928}{109}(222.0795-139.1391)$$

$\bar{d} = 0.030476 \times 82.9404$

$\bar{d} = 2.5$

7.3.10.1 Mean diversity as calculated above is affected both by richness of species and by the distribution of individuals among the species (species composition) and may range from zero to 3.321928 log N. Since the calculated value of mean diversity is a result of the interaction of two parameters which may vary independently, it is often insensitive to subtle changes in community structure. Therefore, unless the environment has been grossly modified, mean diversity ($\bar{d}$) often has limited value in detecting alterations in community structure and serves mainly as an intermediate step in the calculation of a single numerical value for species composition.

7.3.11 To evaluate the component of diversity due to the distribution of individuals among the species (species composition), the calculated $\bar{d}$ must be compared with a hypothetical maximum $\bar{d}$ based on an arbitrarily selected distribution. The measure of redundancy proposed by Margalef (1957) is based on the ratio between $\bar{d}$ and a hypothetical maximum computed as though all species were equally abundant. In nature, equality of species is quite unlikely, so Lloyd and Ghelardi (1964), proposed the term "equitability" and compared $\bar{d}$ with a maximum based on the distribution obtained from MacArthur's (1957) broken stick model. The MacArthur model results in a distribution quite frequently observed in nature; one with a few relatively abundant species and increasing numbers of species represented by only a few individuals. Sample data are not expected to conform to the MacArthur model, since it is only being used as a yardstick against which the distribution of abundances is being compared. Lloyd and Ghelardi (1964) devised a table for determining equitability by comparing the number of species (s) in the sample with the number of species expected (s′) from a community that conforms to the MacArthur model. In the table (reproduced as Table 24) the proposed measure of equitability is:

$$e = \frac{s'}{s}$$

where s = the number of taxa in the sample and s′ = the tabulated value.

7.3.11.1 For the example given above:

$$e = \frac{s'}{s} = \frac{8}{10} = 0.8$$

where "s′" is found from Table 24 using $\bar{d}$ of 2.5. Equitability "e", as calculated, may range from 0 to 1 except in the unusual situation where the distribution in the sample is more equitable than the distribution resulting from the MacArthur model. Such an eventuality will result in values of "e" greater than 1, and this occasionally occurs in samples containing only a few specimens with several taxa represented. The value of "e" is not entirely sample size independent and should not be used for samples containing fewer than five taxa.

7.3.11.2 Equitability ("e") is very sensitive to slight changes in community structure. Since the sample is a representation of the community sampled, a usable index must be sensitive to sample differences and within station variability must be handled by proper study design and adequate replication. Equitability above 0.5 is indicative of waters not affected by oxygen demand wastes. Even slight levels of degradation have been found to reduce equitability below 0.5, generally below 0.3.

7.3.12 Quantitative data can also be produced using the biotic index described in 7.2.6 as long as quantitative methods were used in sample collection and analysis, and proper assumptions are made concerning the subjective nature of the pollution tolerance values.

7.3.13 A rather simple technique for evaluating quantitative data is the sequential comparison index (SCI) which estimates relative differences in biological diversity (Cairns and Dickson, 1971). The method requires no taxonomic expertise on the part of the investigator and is based on differences in the shape, color, and size of the organisms. It should be stressed that the method is useful only as a technique to evaluate the diversity of the bottom community rapidly producing numerical data which can be interpreted statistically. However, it should not be used to replace other more exact techniques providing information on the identity and pollution tolerance of the organisms and requiring persons trained in aquatic ecology.

7.3.14 Wilhm's Species Diversity Index (Wilhm and Dorris, 1968) is based upon information theory and is an attempt to give a numerical value to the environmental changes caused by waste dischargers. This index takes into account not only the number of species encountered, but also the relative abundances of the different species and is very similar to that described in section 7.3.10. Results from this system indicate that values of "d" less than one are indicative of heavy pollution, values from one to three indicate moderate pollution and values above three are found in clean water areas.

7.3.15 Harkins and Austin (1973) have also developed a method that appears to be universal in scope and has worked well in diverse situations. This method is based on average diversity per individual and redundancy which are reduced to a single index value per sample utilizing a nonparametric discrimination technique which then gives a unique distance value from a predefined "biological desert" condition (control values). This condition exists as the case of no organisms present or only one species containing "n" organisms.

7.3.15.1 Computer programs have been written to perform the needed calculations as well as the analysis of variance which can be used with this method. Harkin and Austin's method then is essentially an objective method for reducing several biological indexes to a single meaningful value that will reflect subtle changes in the structure of aquatic communities. The resulting sets of standardized distance values can be compared subjectively or can be subjected to statistical evaluation and probability level of differences assessed. With this method any changes of quality will be detected and can be plotted for long-term trend analysis.

7.4 Rapid Bioassessment Techniques

7.4.1 Rapid Bioassessment Techniques (Plafkin *et al*., 1989) are generally considered both qualitative and semi-quantitative. The protocols were established as a rapid means of detecting aquatic life impairments and assessing their relative severity and are not intended to replace traditional biomonitoring methods. The three protocols each consist of three basic components: water quality/physical characteristics, habitat assessment, and a biosurvey. The biological assessment in each protocol involves an integrated analysis of both functional and structural components of the aquatic communities through use of metrics for benthic macroinvertebrates and fish.

7.4.1.1 Rapid Bioassessment Protocol I consists of an estimation of the level of diversity of the aquatic biota; an estimation of the relative abundance of major macrobenthic taxa, using a qualitative sampling process to include as many habitats as possible; observations of the presence of fish, plants and physical structures; observations on habitat alterations; and observation on possible sources of impact.

7.4.1.2 Rapid Bioassessment Protocol II consists of an in the field estimation of the abundance level of the major aquatic biota, a list of families found in a 100-organisms subsample based on field identification, the number of individuals in each family, and separation of these into scraper and filtering collector functional feeding groups, collection of a course particulate organic material (CPOM) sample, and observations as in Protocol I.

7.4.1.3 Rapid bioassessment Protocol III is similar to Protocol II except that the subsampling and identifications are done in the laboratory and the organisms are identified to genus or species.

7.4.1.4 Rapid Bioassessment Protocols IV and V are based on fish surveys conducted by fishery personnel usually with assistance from the aquatic biologist involved with Protocols I to III.

7.5 Community Metrics and Pollution Indicators

7.5.1 Biological impairment of the benthic community may be assessed by use of metrics including community, population and functional parameters. Metrics measure different components of the community structure and have different ranges of sensitivity to stress. It is advisable, therefore, to use several metrics because an integrated approach provides more assurance of a valid assessment. A few of the more useful metrics are briefly described.

7.5.2 Species (or Taxa) Richness reflects the health of the community through a measurement of the variety of taxa (total number of families and/or genera and/or species) present. Richness generally increases with increasing water quality, habitat diversity, and/or habitat suitability. Sampling of highly similar habitats will reduce the variability in this metric attributable to factors such as current speed and substrate type. Some pristine headwater streams may be naturally unproductive, supporting only a very limited number of taxa. In these situations, organic enrichment may result in an increase in number of taxa.

7.5.3 The modified Hilsenhoff Biotic Index (HBI) (Plafkin et al. 1989) was developed to summarize overall pollution tolerance of the benthic arthropod community with a single value. This index was developed as a means of detecting organic pollution in communities inhabiting rock or gravel riffles/runs. Although Hilsenhoff's (1977) biotic index using tolerance values of 0-5 was originally developed for use in Wisconsin, it is successfully used by several states and should prove reliable for extensive use, perhaps requiring regional modification in some instances. Based on an in depth study of 53 Wisconsin streams Hilsenhoff (1988a) expanded the scale for tolerance values to 0-10. The 0-10 scale was adopted for use with the Rapid Bioassessment Protocol III and was modified to include non-arthropod species.

7.5.3.1 Although it may be applicable for other types of pollutants, use of the HBI in detecting non-organic pollution effects has not been thoroughly evaluated. The state of Wisconsin is conducting a study to evaluate the ability of Hilsenhoff's index to detect non-organic effects. Winget and Mangum (1979) have developed a tolerance classification system applicable to the assessment of nonpoint source impact.

7.5.3.2 Invertebrate Community Index (ICI)--Ohio EPA (1989) measures the condition of the macroinvertebrate community by use of the Invertebrate Community Index (ICI). This index is a modification of the Index of Biotic Integrity (IBI) used for fish (Karr, 1981) consisting of ten community metrics. Scoring of each metric varies with drainage area and ecoregion (Ohio EPA, 1987), and all but one metric is generated from artificial substrate (multiplate) samplers. Metric 10 is based solely on qualitative sample data.

7.5.4 Ratio of Scraper and Filtering Collector Functional Feeding Groups reflect the riffle/run community food base and provides insight into the nature of potential disturbance factors. The proportion of the two feeding groups is important because predominance of a particular feeding type may indicate an unbalanced community responding to an overabundance of a particular food source. The predominant feeding strategy reflects the type of impact detected.

7.5.4.1 A description of the functional feeding group concept can be found in Cummins (1973). Genus-level functional feeding group designations for most aquatic insects can be found in Merritt and Cummins (1984). Within a functional feeding group individual taxa may be either specialists which are restricted to the utilization of a specific food resource or be facultative and thus be able to exploit a broader range of food resources. The trophic generalists (see Merritt and Cummins, 1984) are expected to be better able to tolerate disturbance to aquatic habitats and thus become numerically dominant because of their more flexible ability to utilize available resources.

7.5.4.2 The relative abundance of scrapers and filtering collectors in the riffle/run habitat provides an indication of the periphyton community composition and availability of suspended fine particulate organic material (FPOM) associated with organic enrichment. Scrapers increase with increased abundance of diatoms and decrease as filamentous algae and aquatic mosses (which cannot be efficiently harvested by scrapers) increase. However, filamentous algae and aquatic mosses provide good attachment sites for filtering collectors, and the organic enrichment often responsible for over abundance of filamentous algae provide FPOM utilized by the filterers.

7.5.4.3 Filtering collectors are also sensitive to toxicants bound to fine particles and may decrease in abundance when exposed to sources of such bound toxicants. The scraper-to-filtering-collector ratio may not be a good indication of organic enrichment if adsorbing toxicants are present. This situation is often associated with point source discharges where certain toxicants adsorb readily to dissolved organic matter forming FPOM during flocculation. Toxicants thus become available to filterers via FPOM.

7.5.5 Ratio of Shredder Functional Feeding Group and Total Number of Individuals collected in a coarse particulate organic material (CPOM) sample is also based on the functional feeding group concept. The abundance of the shredder functional group relative to the abundance of all other functional groups allows evaluation of potential impairment as indicated by the CPOM-based shredder community. Shredders are sensitive to riparian zone impacts and are particularly good indicators of toxic effects when the toxicants involved are readily adsorbed to the CPOM and either affect the microbial communities colonizing the CPOM or the shredders directly (Plafkin et al. 1989).

7.5.5.1 The degree a toxicant effects shredders versus filterers depends on the nature of the toxicant and the organic particle adsorption efficiency. Generally, as the size of the particle decreases, the adsorption efficiency increases as a function of the increased surface to volume ratio (Hargrove 1972). Toxicants of a terrestrial source (pesticides and herbicides) accumulate on CPOM prior to leaf fall thus having a substantial effect on shredders. The focus of this approach is on a comparison to the reference community, which should have an abundance and diversity of shredders representative of the particular area under study. This allows for an examination of shredder or collector "relative" abundance as indicators of toxicity.

7.5.6 Ratio of Ephemeroptera-Plecoptera-Trichoptera (EPT) and Chironomidae abundance uses relative abundance of these indicator groups as a measure of community balance. Good biotic condition is reflected in communities having a fairly even distribution among all four major groups and with substantial representation in the sensitive groups Ephemeroptera, Plecoptera, and Trichoptera. Skewed populations having a disproportionate number of the generally tolerant Chironomidae relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987). Certain species of some genera such as Cricotopus are highly tolerant (Lenat, 1983; Mount et al., 1984), opportunistic,and may become numerically dominant in habitats exposed to metal discharges where EPT taxa are not abundant, thereby providing a good indicator of toxicant stress (Winner et al., 1980; Clements et al., 1988).

7.5.6.1 Chironomids tend to become increasingly dominant in terms of percent taxonomic composition and relative abundance along a gradient of increasing enrichment or heavy metals concentration (Ferrington 1987).

7.5.7 The EPT Index (the total number of distinct taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera) compared to total taxa present generally increases with increasing water quality. This value summarizes taxa richness within the insect orders that are generally considered to be pollution sensitive. Headwater streams which are naturally unproductive may experience an increase in taxa (including EPT taxa) in response to organic enrichment.

7.5.8 An alternative to the ratio of EPT and Chironomidae abundance metric is the Indicator Assemblage Index (IAI) developed by Shackleford (1988). The IAI integrates the relative abundances of the EPT taxonomic groups and the relative abundances of chironomids and annelids upstream and downstream of a pollution source to evaluate impairment. The IAI may be a valuable metric in areas where the annelid community may fluctuate substantially in response to pollutant stress.

7.5.9 Percent Contribution of Dominant Taxon to the total number of organisms is an indication of community balance at the lowest possible taxonomic level. (The lowest positive taxonomic level is assumed to be genus or species in most instances). A community dominated by relatively few species would indicate environmental stress. Shackleford (1988) has modified this metric to reflect "dominants in common" (DIC) utilizing the dominant five taxa at the stations of comparison. The DIC will provide a measure of replacement or substitution between the reference community and the downstream station.

7.5.10 Community Similarity Indices are used in situations where reference communities exist. The reference community can be derived through sampling an upstream station or prediction for a region using a reference data base. Data sources or ecological data files may be available to establish a reference community for comparison. Several of the many similarity indices available are discussed below:

7.5.10.1 Community Loss Index measures the loss of benthic species between a reference station and the station of comparison. The community loss index was developed by Courtemanch and Davies (1987) and is an index of dissimilarity with values increasing as the degree of dissimilarity from the reference station increases. Values range from zero (0) to "infinity." Based on preliminary data analysis, this index provides greater discrimination than the following two community similarity indices. The formula for determining community loss index is:

$$I = \frac{a - c}{b}$$

where I = Coefficient of Community Loss, "a" is the number of taxa at the unimpacted site, "b" is the number of taxa at the study site, and "c" is the taxa common to "a" and "b". The result is a ratio of the number of taxa assumed lost due to the pollution source (a-c) to the number of taxa remaining including any new taxa.

7.5.10.2 Jaccard Coefficient of Community measures the degree of similarity in taxonomic composition between two stations in terms of taxa presence or absence and discriminates between highly similar collections (Jaccard, 1912). Coefficient values, ranging from 0 to 1.0, increase as the degree of similarity with the reference station increases. See Boesch (1977), and USEPA (1983) for more detail. The formula for the Jaccard Coefficient is:

$$\text{Jaccard Coefficient} = \frac{a}{a + b + c}$$

where

a = number of species common to both samples
b = number of species present in Sample B but not A
c = number of species present in Sample A but not B

Sample A = reference station
Sample B = station of comparison

7.5.10.3 The Index of Similarity (S) Between Two Samples has been used to determine whether shifts in community assemblages have occurred along a stream gradient or above and below a pollutional impact. The Index of Similarity can also be used as a quality assurance tool when evaluating variance in community assemblages between two control or reference sites. The inverse of the Index of Similarity is known as the Index of Dissimilarity. Both are reported as percentages and the formula is (Odum, 1971):

$$S = \frac{2C}{A + B}$$

Where A = Number of Species in Sample 1
B = Number of Species in Sample 2
C = Number of Species Common to both Species
1 - S = Index of Dissimilarity

7.5.10.4 The Pinkham and Pearson Community Similarity Index measures the degree of similarity in taxonomic composition in terms of taxa abundances and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant species. See Pinkham and Pearson (1976) and USEPA (1983) for more detail. The formula is:

$$S.I._{ab} = \Sigma \; \frac{\text{Min}\ (X_{ia}, X_{ib})}{\text{Max}\ (X_{ia}, X_{ib})} \left[\begin{array}{c} \frac{X_{ia}}{X_a} \cdot \frac{X_{ib}}{X_b} / 2 \\ \text{Weighting factor} \end{array} \right]$$

where X_{ia}, X_{ib} = number of individuals in the i^{th} species in sample A or B.

7.5.10.5 A Percent Similarity Method described by Gauch and Whittaker (1972) matches the benthic community structure of the site under study with an unimpacted site (control). It is a calculation of the degree to which the distribution of individuals within specific taxa in one site is similar to the distribution in another matched site. The value may range from zero (0) for sites with no taxa in common, to one (1) for identical communities.

$$P.S. = \frac{2 \; \Sigma \quad \text{min.}\ (P_{ij}, P_{ik})}{(P_{ij} + P_{ik})}$$

where P.S. = Percent similarity, P_{ij} = Percentage of taxa "i" in community "j", and P_{ik} = Percentage of organisms of taxa "i" in community "k".

7.5.10.6 Other Community Similarity Indices include Spearman's Rank Correlation (Snedecor and Cochran, 1980); Moriseta's Index (Moriseta, 1959); Biotic Condition Index (Winget and Mangum, 1979); and Bray-Curtis Index (Bray and Curtis, 1957; Whittaker, 1952). Calculation of a chi-square "goodness of fit" (Cochran, 1952) may also be appropriate.

7.5.11 Presence and/or Absence of Specific Indicator Organisms is usually based upon a classification of organisms as either pollution sensitive (intolerant), facultative (variable), or tolerant (see paragraph 7.2.5). For example, usually stoneflies, mayflies, and caddisflies are considered sensitive or facultative and, therefore, are usually the first to suffer in a polluted environment. Sludgeworms and bloodworms, on the other hand, can tolerate very heavy pollutional loads.

7.5.11.1 The method differs from the biotic index of Hilsenhoff (1977, 1987) in that only selected indicator species are used to make decisions, whereas his biotic index used all the organisms in the samples.

7.5.11.2 A classic example of a system using the presence/absence criteria, is the Saprobien system (Kolkwitz and Marsson, 1908) which recognizes three basic zones of pollution ranging from a zone of heavy pollution (polysaprobic) characterized by a lack of dissolved oxygen, an abundance of bacteria, and the presence of a few tolerant species, to a zone of recovery (oligosaprobic) characterized by relatively pure water with a somewhat stable species diversity and dissolved oxygen concentration. This system was developed for use in Europe. Its usefulness is limited to organic pollutants in slow moving streams and is not always applicable to rivers and streams of the United States. A modification of the method was used in studies of the Illinois River (Richardson, 1928) and of a stream in southern Ohio (Gaufin and Tarzwell, 1956). A further modification of this method in combination with the biotic index was recently used by Rabeni et al. (1985) in the study of a Maine river. The results appear to be encouraging for wide use in this country. This approach is highly subjective and would naturally vary from one stream to another. It is also restricted to organic-type wastes.

7.5.12 Mean Number of Individuals per Sample is a simple means of comparing biological data. All of the individuals in all the replicate samples from one station are counted and divided by the number of replicates to yield the number of individuals per sample.

7.6 **Statistical Methods**

7.6.1 Graphical Examination of Data

Often the most elementary techniques are of the greatest use in data interpretation. Visual examination of data can point the way for more discriminatory analyses, or on the other hand, interpretations may become so obvious that further analysis is superfluous. In either case, graphical examination of data is often the most effortless way to obtain an initial examination of data and affords the chance to organize the data. Therefore, it is often done as a first step. Some commonly used techniques are presented below.

7.6.1.1 Raw Data

It is of utmost importance that raw data be recorded in a careful, logical, interpretable manner together with appropriate, but not superfluous, annotations. Note that although some annotations may be considered superfluous to the immediate intent of the data, they may not be so for other purposes. Any note that might aid in determining whether the data are comparable to other similar data, etc., should be recorded if possible.

7.6.1.2 Frequency Histograms

To construct a frequency histogram (see Freund, 1986) from the data, examine the raw data to determine the range, then establish intervals. Choose the intervals with care so they will be optimally integrative and differentiable. If the intervals are too wide, too many observations will be integrated into one interval and the picture will be hidden; if too narrow, too few will fall into one interval and a confusing overdifferentiation or overspreading of the data will result. It is often enlightening if the same data are plotted with the use of several interval sizes. Construct the intervals so that no doubt exists as to which interval an observation belongs, i.e., the end of one interval must not be the same number as the beginning of the next.

Although a frequency table contains all the information that a comparable histogram contains, the graphical value of a histogram is usually worth the small effort required for its construction. Histograms are more immediately interpretable. The height of each bar is the frequency of the interval; the width is the interval width.

7.6.1.3 Frequency Polygon

Another way to present essentially the same information as that in a frequency histogram is the use of a frequency polygon. Plot points at the height of the frequency and at the midpoint of the interval, and connect the points with straight lines.

7.6.1.4 Cumulative Frequency

Cumulative frequency plots are often useful in data interpretation. The height of a bar (frequency) is the sum of all frequencies up to and including the one being plotted. Thus, the first bar will be the same as the frequency histogram, the second bar equals the sum of the first and second bars of the frequency histogram, etc., and the last bar is the sum of all frequencies.

Closely related to the cumulative frequency histogram is the cumulative frequency distribution graph, a graph of relative frequencies. To obtain the cumulative graph, merely change the scale of the frequency axis on the cumulative frequency histogram. The scale change is made by dividing all values on the scale by the highest value on the scale.

The value of the cumulative frequency distribution graph is to allow relative frequency to be read, i.e., the fraction of observations less than or equal to some chosen value. Exercise caution in extrapolating from a cumulative frequency distribution to other situations. Always bear in mind that in spite of a planned lack of bias, each sample, or restricted set of samples, is subject to influences not accounted for and is therefore unique. This caution is all the more pertinent for cumulative frequency plots because they tend to smooth out some of the variation noticed in the frequency histogram. In addition, the phrase "fraction of observations less than or equal to some chosen value" can easily be read "fraction of time the observation is less than or equal to some chosen value." It is tempting to generalize from this reading and extend these results beyond their range of applicability.

7.6.1.5 Two-dimensional Graphs

Often data are taken where the observations are recorded as a pair (biomass and nutrient concentration). Here a quick plot of the set of pairs will usually be of value. The peaks and troughs, their frequency, together with intimate knowledge of the conditions of the study, might suggest something of biological interest, further statistical analysis, or further field or laboratory work.

7.6.1.6 In summary, carefully prepared tables and graphs may be important and informative steps in data analysis. The added effort is usually small, whereas gains in interpretive insight may be large. Therefore, graphic examination of data is a recommended procedure in the course of most investigations.

7.6.2 Sample Mean and Variance

7.6.2.1 Notation

Knowledge of certain computations and computational notations is essential to the use of statistical techniques. Some of the more basic of these will be briefly reviewed here.

To illustrate the computations, let us assume we have a set of data, i.e., a list of numeric values written down. Each of these values can be labeled by a set of numerals beginning with 1. Thus, the *first* of these values can be called X_1, the *second* X_2, etc., and the *last* one we call X_n,. The data values are labeled with consecutive numbers (recall from the definitions that these numeric values are observations), and there are n values in the set of data. A typical observation is X_i, where i may take any value between 1 and n, inclusive, and the subscript indicates which X is being referenced.

The sum of the numbers in a data set, such as our sample, is indicated in statistical computations by capital sigma, Σ. Associated with Σ are an operand (here, X_i), a subscript (here, i = 1), and a superscript (here, n).

$$\sum_{i=1}^{n} X_i$$

The subscript i= 1 indicates that the value of the operand X is to be the number labeled X_i in our data set and that this is to be the first observation of the sum. The superscript n indicates that the last number of the summation is to be the value of X_n the last X in our data set.

7.6.2.2 Calculation of the Sample Mean and Variance

Computations for the mean, variance, standard deviation, variance of the mean, and standard deviation of the mean (standard error) are presented below. Note that these are computations for a sample of n observations, i.e., they are statistics.

Note: The X_i's are squared, then the summation is performed in the first term

$$\text{Mean } (\overline{X}): \quad \overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$$\text{Variance } (s^2): \quad s^2 = \frac{\sum_{i=1}^{n} X_i^2 - \frac{(\sum_{i=1}^{n} X_i)^2}{n}}{n-1}$$

of the numerator; in the second term, the sum of the X_i's is first formed, then the sum is squared, as indicated by the parenthesis.

$$\text{Standard deviation } (s): \quad s = \sqrt{s^2}$$

$$\text{Variance of the mean } (s_{\overline{X}}^2): \quad s_{\overline{X}}^2 = \frac{s^2}{n}$$

$$\text{Standard deviation of the mean } (s_{\overline{X}}): \quad s_{\overline{X}} = \sqrt{s_{\overline{X}}^2} = \frac{s}{\sqrt{n}}$$

7.6.3 Rounding

The questions of rounding and the number of digits to carry through the calculations always arise in making statistical computations. Measurement data are approximations, since they are rounded when the measurements were taken; count data and binomial data are not subject to this type of approximation.

Observe the following rules when working with measurement or continuous data.

* When rounding numbers to some number of decimal places, first look at the digit to the right of the last place to be retained. If this number is greater than 5, the last place to be retained is rounded up by 1; if it is less than 5, do not change the last place - merely drop the extra places. To round to 2 decimal places:

Unrounded	Rounded
1.239	1.24
28.5849	28.58

- If the digit to the right of the last place to be retained is 5,then look at the second digit to the right of the last place to be kept, provided that the unrounded number is recorded with that digit as a significant digit. If the second digit to the right is greater than 0, then round the number up by 1 in the last place to be kept; if the second digit is 0, then look at the third digit, etc. To round to 1 place:

Unrounded	Rounded
13.251	13.3
13.25001	13.3

- If the number is recorded to only one place to the right of the last place to be kept, a special rule (odd-even rule) is followed to ensure that upward rounding occurs as frequently as downward rounding. The rule is: if the digit to the right of the last place to be kept is 5, and is the last digit of significance, round up when the last digit to be retained is *odd* and drop the 5 when the last digit to be retained is even. To round to 1 place:

Unrounded	Rounded
13.25	13.2
13.3500	13.4

Caution: all rounding must be made in 1 step to avoid introducing bias. For example the number 5.451 rounded to a whole number is clearly 5, but if the rounding were done in two steps it would first be rounded to 5.5 then 6.

Retention of significant figures in statistical computations can be summarized in three rules:

- Never use more significance for a raw data value than is warranted.
- During intermediate computations keep all significant figures for each data value, and carry the computations out in full.
- Round the final result to the accuracy set by the least accurate data value.

7.6.4 Tests of Hypotheses

7.6.4.1 Introduction

Often in biological field studies some aspect of the study is directed to answering a hypothetical question about a population (Allan, 1984). If the hypothesis is quantifiable, such as: "At the time of sampling, the standing crop of macroinvertebrates per basket at station 1 was the same as at station 2", then the hypothesis can be tested statistically. The question of drawing a sample in such a way that there is freedom from bias, so that such a test may be made, was discussed in Section 4, Selection of Sampling Stations.

There are many different types of hypothesis tests. Two basic categories of hypothesis tests are parametric tests, those based on the data following a specific distribution, and nonparametric tests, those based on relative rankings of the data. Three standard parametric tests of hypotheses will be presented here: the t-test, the χ^2 test, and the F-test. For information concerning nonparametric tests see Conover, 1980.

7.6.4.2 T-test

The t-test is used to compare a sample statistic (such as the mean) with some value for the purpose of making a judgment about the population as indicated by the sample. The comparison value may be the mean of another sample (in which case we are using the two samples to judge whether the two populations are the same). The form of the t-statistic is

$$t = \frac{\phi - \theta}{s_\phi}$$

where ϕ = some sample statistic; S_ϕ = the standard deviation of the sample statistic; and θ = the value to which the sample statistic is compared (the value of the null hypothesis).

The use of the t-test requires the use of t-tables. The t-table is a two-way table usually arranged with the column headings being the probability, α, of rejecting the null hypothesis when it is true, and the row headings being the degrees of freedom. Entry of the table at the correct probability level requires a discussion of two types of hypotheses testable using the t-statistic.

The null hypothesis is a hypothesis of no difference between a population parameter and another value. Suppose the hypothesis to be tested is that the mean, μ, of some population equals 10. Then we would write the null hypothesis (symbolized H_o) as

$$H_o: \mu = 10$$

Here 10 is the value of θ in the general form for the t-statistic. An alternative to the null hypothesis is now required. The investigator, viewing the experimental situation, determines the way in which this is stated. If the investigator merely wants to answer whether the sample indicates that μ = 10 or not, then the alternate hypothesis, H_a, is

$$H_a: \mu \neq 10$$

If it is known, for example, that μ cannot be less than 10, the H_a is

$$H_a: \mu > 10$$

and by similar reasoning the other possible H_a is

$$H_a: \mu < 10$$

Hence, there are two types of alternate hypotheses: one where the alternative is simply that the null hypothesis is false H_a: $\mu \neq 10$; the other, that the null hypothesis is false and, in addition, that the population parameter lies to one side or the other of the hypothesized value [H_a : μ (> or <) 10]. In the case of H_a: $\mu \neq 10$, the test is called a two-tailed test; in the case of either of the second types of alternate hypotheses,, the t-test is called a one-tailed test.

To use a t-table, it must be determined whether the column headings (probability of a larger value, or percentage points, or other means of expressing α) are set for one-tailed or two-tailed tests. Some tables are presented with both headings, and the terms "sign ignored" and "sign considered" are used. "Sign ignored" implies a two-tailed test, and "sign considered" implies a one-tailed test. Where tables are given for one-tailed tests, the column for any probability (or percentage) is the column appropriate to twice the probability for a two-tailed test. Hence, if a column heading 0.025 and the table is for one tailed tests, use this same column for 0.05 in a two tailed test (double any one-tailed test heading to get the proper two-tailed test heading; or conversely, halve the two-tailed test heading to obtain proper headings for one-tailed tests).

Testing H_o : $\mu = M$ (the population mean equals some value M):

$$t = \frac{\bar{X} - M}{s_{\bar{X}}}$$

where $\bar{X}$ is given by the sample mean; M = the hypothesized population mean; and $s_{\bar{X}}$ is given by the standard deviation (standard error) of the mean. The t-table is entered at the chosen probability level (often 0.05) and n-1 degrees of freedom, where n is the number of observations in the sample.

When the computed t-statistic exceeds the tabular value there is said to be a $1-\alpha$ probability that H_o is false.

Testing H_o: $\mu_1 = \mu_2$ (the mean of the population from which sample 1 was taken equals the mean of the population from which sample 2 was taken):

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_{\overline{X}_1} - s_{\overline{X}_2}}$$

where $\overline{X}_1$ and $\overline{X}_2$ are the means from sample 1 and sample 2 respectively and $s_{\overline{X}_1} - s_{\overline{X}_2}$ is the standard error for the difference $\overline{X}_1-\overline{X}_2$ calculated as follows:

$$s_{\overline{X}_1 - \overline{X}_2} = \sqrt{\frac{(n_1-1)\,s_1^2 + (n_2-1)\,s_2^2}{(n_1+n_2-2)} \cdot \left(\frac{n_1+n_2}{n_1 \cdot n_2}\right)}$$

where s_1^2 and s_2^2 are variances of samples one and two respectively, and n_1 and n_2 are the number of observations for each sample.

For all conditions to be met where the t-test is applicable, the sample should have been selected from a population distributed as a normal distribution. Even if the population is not distributed normally, however, as sample size increases, the t-test approaches to applicability. If it is suspected that the population deviates too drastically from the normal, exercise care in the use of the t-test. Another assumption of the t-test is that the variances of the two populations are equal. Both the normality assumption and the equal variance assumption should be formally tested prior to using the t-test.

7.6.4.3 Chi-Square Test (χ^2)

The chi-square test is useful for statistically testing a hypothesis. Like t, χ^2 values may be found in mathematical and statistical tables tabulated in a two-way arrangement. Usually, the column headings are probabilities of obtaining a larger χ^2 value when H_o is true, and the row headings are degrees of freedom.

If the calculated χ^2 exceeds the tabular value, then the null hypothesis is rejected. The chi square test is often used with the assumption of approximate normality in the population.

Chi-Square appears in two forms that differ not only in appearance, but that provide formats for different applications.

One form is useful in tests regarding hypotheses about σ^2:

$$\chi^2 = \frac{(n-1)\,s^2}{\sigma_o^2} \quad \text{where } H_o : \sigma^2 = \sigma_o^2$$

The other form:

$$\chi^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

where O = an observed value, and E = an expected (hypothesized) value, is especially useful in sampling from binomial and multinomial distributions, i.e., where the data may be classified into two or more categories (k).

Consider first a binomial situation. Suppose the Stenonema mayflies (2 species) from three stream riffle stations are pooled and the hypothesis of an equal ratio of the two species is tested based on the hypothetical data in Table 9.

Table 9. POOLED STENONEMA DATA FROM THREE RIFFLE STATIONS

Stenonema sp. 1	Stenonema sp. 2	Total
892* (919**)	946* (919**)	1838

* Observed value.
** Expected or hypothesized value.

To compute the hypothesized values (919 above) it is necessary to have formulated a null hypothesis. In this case it was H_o:No. Sp. 1 = No. Sp. 2 = (0.5) (Total).

Expected values are always computed based upon the null hypothesis. The computation for χ^2 is

$$\chi^2 = \frac{(892-919)^2 + (946-919)^2}{919} = 1.59\ n.s.^{*}$$

[*] n.s. = not significant at $\alpha = 0.05$

There is one degree of freedom for this test. Since the computed χ^2 is not greater than the tabulated χ^2 (3.84) for $\alpha = 0.05$, the null hypothesis is not rejected. This test, of course, applies equally well to data that has not been pooled, i.e., where the values are from two unpooled categories.

The information contained in each of the collections is partially obliterated by pooling. If the identity of the collections is maintained, two types of tests may be made; a test of the null hypothesis for each collection separately; and a test of interaction, i.e., whether the ratio depends upon the riffle from which the sample was obtained (Table 10).

With the use of the same null hypothesis, the following results are obtained. All tests were made at the $\alpha = 0.01$ level of significance. (Note: A significance level of 0.01 is used, instead of 0.05, to allow for the fact that multiple tests are being made within one experiment)

The individual χ^2's were computed, using the second form of chi square above, in separate tests of the hypothesis for each riffle. Note that the first two are not significant whereas the third is significant. This points to probable ecological differences among riffles, a possibility that would not have been discerned by pooling the data.

Table 10. STENONEMA DATA FROM THREE RIFFLE STATIONS

Riffle	Sp. 1	Sp. 2	Total	χ^2
1	346* (354)+	362 (354)	708	0.36 n.s.
2	302 (288)	274 (288)	576	1.30 n.s.
3	244 (277)	310 (277)	554	7.88
Total	892 (919)	946 (919)	1838	1.59 n.s.

* Observed values.
+ Expected, or hypothesized values.

The test for interaction (dependence) is made by summing the individual χ^2's and subtracting the χ^2 obtained using totals, i.e.,

$$\chi^2 \text{ (interactions)} = \Sigma \chi^2 \text{ (individuals)} - \chi^2 \text{ (total)}$$

$$= 0.36 + 1.30 + 7.88 - 1.59$$

$$= 7.95$$

The degrees of freedom for the interaction χ^2 are the number of individual χ^2's minus one; in this case, two. This interaction χ^2 is significant, which indicates that the dominant species is indeed dependent upon the riffle.

Another χ^2 test may be illustrated by the following example. Suppose that comparable techniques were used to collect from four streams. With the use of three species common to all streams, it is desired to test the hypothesis that the three species occur in the same ratio regardless of stream, i.e., that their ratio is independent of stream (Table 11).

TABLE 11. OCCURRENCE OF THREE SPECIES OF MIDGES

Stream	Number of organisms			Frequency
	Species 1	Species 2	Species 3	
1	24* (21.7)+	12 (12.5)	30 (31.7)	66
2	15 (18.5)	14 (10.6)	27 (26.9)	56
3	28 (27.4)	15 (15.7)	40 (39.9)	83
4	20 (19.4)	9 (11.2)	30 (28.4)	59
Total	87	50	127	264
Expected ratio	87/264	50/264	127/264	

* Observed values.
+ Expected or hypothesized.

To discuss the table above, O_{ij} = the observation for the i^th stream and the j^th species. Hence, O_{23} is the observation for stream two and species three. A similar indexing scheme applies to the expected values, E_{ij}. For the totals, a subscript replaced by a dot $E_{i.}$ symbolizes that summation has occurred for the observations indicated by that subscript. Hence, $O_{.2}$ is the total for species two (50); $O_{3.}$ is the total for stream three (83); and $O_{..}$ is the grand total (264).

Computations of expected values make use of the null hypothesis that the ratios are the same regardless of stream. The best estimate of this ratio for any species is $O_{.j}/O_{..}$, the ratio of the sum for species j to the total of all species. This ratio multiplied by the total for stream i gives the expected number of organisms of species j in stream i:

$$E_{ij} = \frac{O_{\cdot j}}{O_{\cdot\cdot}} \cdot (O_{i\cdot})$$

For example,

$$E_{12} = \frac{O_{\cdot 2}}{O_{\cdot\cdot}} \cdot (O_{1\cdot}) = \frac{50}{264} \cdot (66) = 12.5$$

χ^2 is computed as

$$\chi^2 = \sum_i \sum_j \frac{(O_{ij} - E_{ij})^2}{E_{ij}} = 2.69 \quad (n.s.)$$

For this type of hypothesis, there are (rows - 1) (columns - 1) degrees of freedom, in this case

$$(4-1)\ (3-1) = 6$$

In the example, since the computed χ^2 is not greater than the tabulated χ^2(12.59) for α=0.05 the null hypothesis cannot be rejected. Thus, there is no evidence that the ratios among the organisms are different for different streams.

Tests of two types of hypotheses by χ^2 have been illustrated. The first type of hypothesis was one where there was a theoretical ratio, i.e., the ratio of sp.1 to sp.2 is 1:1. The second type of hypothesis was one where equal ratios were hypothesized, but the values of the ratios themselves were computed from the data. To draw the proper inference, it is important to make a distinction between these two types of hypotheses.

7.6.4.4 Analysis of Variance

Another form of hypothesis testing is the analysis of variance (ANOVA). The ANOVA is a powerful and general technique applicable to data from virtually any experimental or field study. There are restrictions, however, in the use of the technique. Experimental errors are assumed to be normally (or approximately normally) distributed about a mean of zero and have a common variance; they are also assumed to be independent (i.e., there should be no correlations among responses that are unaccounted for by the identifiable factors of the study or by the model). The effects tested must be assumed to be linearly additive. In practice these assumptions are rarely completely fulfilled, but the analysis of variance can be used unless significant departures from normality, or correlations among adjacent observations, or other types of measurement bias are suspected. It would be prudent, however, to check with a statistician regarding any uncertainties about the applicability of the test before issuing final reports or publications. Two simple but potentially useful examples of the analysis of variance are presented to illustrate the use of this technique.

7.6.4.4.1 Randomized Design

The analysis of variance for completely randomized designs provides a technique often useful in field studies. This test is commonly used for data derived from highly-controlled laboratory or field experiments where treatments are applied randomly to all experimental units, and the interest lies in whether or not the treatments significantly affected the response of the experimental units. This case may be of use in water quality studies, but in these studies the treatments are the conditions found, or are classifications based upon ecological criteria. Here the desire is to detect any differences in some type of measurement that might exist in conjunction with the field situation or the classifications or criteria.

For example, suppose it is desired to test whether the biomass of organisms in drift nets in a stream varies due to sampling time. Data from such a study are presented in Table 12.

In testing with the analysis of variance, as with other methods, a null hypothesis should be formulated. In this case the null hypothesis could be:

H_o: There are no differences in the biomass of organisms that may be attributed to time of sampling.

TABLE 12. MACROINVERTEBRATE BIOMASS COLLECTED AT DIFFERENT TIMES OF DAY FROM THE LITTLE MIAMI RIVER AT MILFORD, OHIO

Sampling Time (Time)	Replicate number	Biomass (mg dry wt.)
9:00AM - 1:00PM	1	1678
	2	1211
	3	1644
	4	1137
1:00AM - 4:00PM	1	1604
	2	1639
	3	2077
	4	2581
4:00PM - 7:00PM	1	4276
	2	2400
	3	3183
	4	3451

In utilizing the analysis of variance, the test for whether there are differences across time is made by comparing two types of variances, most often called "mean squares" in this context. Two mean squares are computed: one based upon the means for times; and one that is free of the effect of the means. In our example, a mean square for times is computed with the use of the averages (or totals) from the sampling time. The magnitude of this mean square is affected both by differences among the means and by differences among nets of the same time. The mean square within time is computed that has no contribution due to time differences. If the null hypothesis is true, then differences among sampling time do not exist and, therefore, they make no contribution to the mean square for times. Thus, both mean squares (between times and within times) are estimates of the same variance, and with repeated sampling, they would be expected to average to the same value. If the null hypothesis (H_0) is true, the ratio of these values is expected to equal one. If H_0 is not true, i.e., if there are real differences due to the effect of times, then the mean square between times is affected by these differences and is expected to be the larger. The ratio in the second case is expected to be greater than one. The ratio of these two variances forms an F-test.

The analysis of variance is presented in Table 13A.

TABLE 13A. Generalized ANOVA Table

Source	df	SS
Total	$N-1$ *	$\sum_i \sum_j X_{ij}^2 - C$
Between Times	$t-1$	$[(\sum_i X_{i\cdot}^2)/r_i] - C$
Within Times	$\sum_i (r_i - 1)$	Total SS – Stream SS

*The symbols are defined as N=total number of observations (nets); t=number of sampling times; r_i=number of nets for sample time i; X_{ij}=an observation (biomass of net j at sampling time i); X_i=sum of the observations for sampling time i; and

C=correction for mean = $\frac{(\sum_{i}\sum_{j} X_{ij})^2}{N}$

TABLE 13B. Completed ANOVA Table Using Macroinvertebrate Biomass Data

Source	df	SS	MS	F
Total	11	10,381,723		
Between Times	2	7,717,020	3,858,510	13.03**
Within Times	9	2,664,703	296,078	

** Significant at the 0.05 probability level.

The computations are:

$$C=\frac{(5670+7901+13310)^2}{12}=60,215,680$$

$$\sum_{i}\sum_{j} X_{ij}^2=(1678)^2+(1211)^2+...+(3451)^2=70,597,403$$

$$Total\ SS=70,597,403-60,215,680=10,381,723$$

$$\sum_{i}\frac{X_{i.}^2}{r_i}=\frac{(5670)^2}{4}-\frac{(7901)^2}{4}+\frac{(13310)^2}{4}=67,932,700$$

$$Between\ Times\ SS=67,932,700-60,215,680=7,717,020$$

$$\begin{aligned} Within\ Times\ SS &= Total\ SS - Between\ Times\ SS \\ &= 10,381,723-7,717,020=2,664,703 \end{aligned}$$

The mean squares (MS column) are computed by dividing the sums of squares (SS column) by its corresponding degrees of freedom (df column). The F-test is performed by computing the ratio, (Between Times MS)/(Within Times MS), in this case:

$$\frac{3,858,510}{296,078} = 13.03$$

When the calculated F value (13.03) is compared with the F values in the table (tabular F values) where df = 2 for the numerator and df = 9 for the denominator, we find that the calculated F exceeds the value of the tabular F for probability greater than 0.95. Thus the conclusion is that there are significant differences in biomass due to time of sampling.

Note that this analysis presumes good biological procedure and obviously cannot discriminate differences in sampling time from differences arising, for example, from the net having been placed in riffles with different current velocity. In general, the form of any analysis of variance derives from a model describing an observation in the experiment. In the example, the model, although not stated explicitly, assumed only one factor affecting a biomass measurement -- sampling time. If the model had included other factors, a more complicated analysis of variance would have resulted.

7.6.4.4.2 Factorial Design

Another application of a simple analysis of variance may be made where the factors are arranged factorially. Suppose a field study was conducted where the effect of a suspected toxic effluent upon the macroinvertebrate fauna of a river above and below a sewage treatment plant (STP) was in question (Tables 14A and 14B). Five samples were taken about one-quarter mile upstream and five one-quarter mile downstream in the spring, and the sampling scheme was repeated again in the summer. Standard statistical terminology refers to each of the combinations P_1T_1, P_2T_1, P_1T_2, and P_2T_2 as treatments or treatment combinations.

In planning for this field study, a null and alternate hypothesis should have been formed. In fact, whether stated explicitly or not, the null hypothesis was:

H_o: The toxic effluent has no effect upon the macroinvertebrate biomass collected.

This hypothesis is not stated in statistical terms and, therefore, only implicitly tells us what test to make. Let us look further at the analysis before attempting to state a null hypothesis in statistical terms.

In this study two factors are identifiable: times and positions. A study could have been done on each of the two factors separately, i.e., an attempt could have been made to distinguish whether there was a difference associated with times, assuming all other factors insignificant, and likewise with the positions. The example, used here, however, includes both factors simultaneously. Data are given for times and for positions but with the complication that we cannot assume that one is insignificant when studying the other. For the purpose of this study, whether there is a significant difference with times or on the other hand with positions, are questions that are of little interest. Of interest to this study is whether the above-below the STP difference varies with times. This type of contrast is termed a positions-times interaction. Thus, our null hypothesis is, in statistical terminology:

H_o: There is no significant interaction effect

An analysis of variance may be used to test this hypothesis. In order to meet the normality and homogeneity of variance assumptions of the analysis, the raw data were $\log_{10}$ transformed (Table 14B). All calculations are on the transformed data.

TABLE 14A. MACROINVERTEBRATE BIOMASS (GRAMS WET WT.)

Time Collected	Collected above STP	Collected below STP
Spring	437	193
	343	86
	337	119
	635	505
	373	171
Summer	888	28
	1778	18
	4332	117
	1078	26
	859	78

TABLE 14B. LOG_{10} TRANSFORMED DATA

Time Collected	Collected above STP	Collected below STP
Spring	2.64	2.28
	2.54	1.93
	2.53	2.08
	2.80	2.70
	2.57	2.23
Summer	2.95	1.45
	3.25	1.26
	3.64	2.07
	3.03	1.41
	2.93	1.89

TABLE 15. TREATMENT TOTALS FOR THE DATA OF TABLE 14B

Total	Positions		Times totals
	Above	Below	
Spring	13.08	11.22	24.3
Summer	15.8	8.08	23.88
	Positions		Grand
totals	28.88	19.3	48.18

Symbolically, an observation must have three indices specified to be completely identified: position, time, and sample number. Thus there are three subscripts: X_{ijk} is an observation at position i, time j, and from sample k. A value of 1 for i is above the STP; 2, below the STP; 1 for j is spring; 2, summer. A particular example is X_{123}, the third sample above the STP for the summer, or 3.64. A total (Table 15) is specified by using the dot notation. For the value of $X_{ij\cdot}$, then the individually sampled values for position i, time j are totaled. It is a total for a treatment combination. For example, the value of $X_{11\cdot}$, is 13.08, and the value of $X_{1\cdot\cdot}$, where sampling and times are both totaled to give the total for above the STP is 28.88. Treatment totals are presented in Table 15.

For a slight advantage in generality, let the following additional symbols apply: t = number of times of sampling (in this case t = 2); p = number of positions sample (in this case p = 2); s = number of samples per treatment combination; and n = the total number of observations.

The computations are:

Correction for the mean (CT):

$$CT = \frac{(\sum_i \sum_j \sum_k X_{ijk})^2}{n} = \frac{(48.18)^2}{20} = 116.06$$

$$TSS = \sum_i \sum_j \sum_k X_{ijk}^2 - CT = (2.64)^2 + (2.54)^2 + \cdots + (1.89)^2 - 116.06 = 7.54$$

Note that the divisor (5) may be factored out here, if desired, but where a different number of samples is taken for each treatment combination it should be left as above.

Position Sum of Squares (SSP):

$$SSP = \frac{(\sum_{i} X_{i..})^2}{st} - CT = \frac{(28.88)^2}{10} + \frac{(19.3)^2}{10} - 116.06 = 4.59$$

Times Sum of Squares (SST):

$$SST = \frac{(\sum_{j} X_{.j.})^2}{sp} - CT = \frac{(24.3)^2}{10} + \frac{(23.88)^2}{10} - 116.06 = 0.01$$

Interaction of Positions and Times of Sums Squares (SSPT):

$$SSPT = \frac{(\sum_{i} \sum_{j} X_{ij.})^2}{s} - SPS - SST - CT$$

$$\frac{(13.08)^2}{5} + \frac{(11.22)^2}{5} + \frac{(15.80)^2}{5} + \frac{(8.08)^2}{5} - 4.59 - 0.01 - 116.06 = 1.72$$

Error Sums of Squares (SSE):

$$SSE = TSS - SSP - SST - SSPT = 7.54 - 4.59 - 0.01 - 1.72 = 1.22$$

The completed ANOVA, including F tests, is given in Table 16. Although not important to this example, the main effects, positions and times, are tested for significance. The F table is entered with df = 1 for effect tested, and df = 16 for error. The positions effect is significant and the times effect is not significant, both tested at α=0.05. The interaction effect is significant, and we, therefore, conclude that there is a significant effect of the effluent changes across time on biomass.

TABLE 16. ANALYSIS OF VARIANCE TABLE FOR FIELD STUDY DATA OF TABLE 14

Source	df	SS	MS	F
Positions	1	4.59	4.59	57.38 **
Times	1	0.01	0.01	0.125
Positions X Times	1	1.72	1.72	21.51 **
Error	16	1.22	0.08	
Total	19	7.54		

** Significant at the 0.05 probability level.

7.6.5 Confidence Interval for Means

When means are computed in field studies, the desire often is to report them as intervals rather than as fixed numbers. This is entirely reasonable because computed means are virtually always derived from samples and are subject to the same uncertainty that is associated with the sample.

The correct computation of confidence intervals requires that the distribution of the observations be known. But very often approximations are close enough to correctness to be of use, and often are, or may be made to be, conservative. For computation of confidence intervals for the mean, the normal distribution is usually assumed to apply for several reasons: the central limit theorem assures us that with large samples the mean is likely to be approximately normally distributed; the required computations are well known and are easily applied; and when the normal distribution is known not to apply, suitable transformation of the data often is available to allow a valid application.

The confidence interval for a mean is an interval within which the true mean is said to have some stated probability of being found. If the probability of the mean not being in the interval is α (α could equal 0.1, 0.05, 0.01, or any probability value), then the statement may be written:

$$P\,(CL_1 < \mu < CL_2) = 1 - \alpha$$

This is read, "The probability that the lower confidence limit (CL_1) is less than the true mean (μ) and that the upper confidence limit (CL_2) is greater than the true mean, equals $1-\alpha$." However, we never know whether or not the true mean is actually included in the interval. So the confidence interval statement is really a statement about our procedure rather than about μ. It says that if we follow the procedure for repeated experiments, a proportion of those experiments equal to α will, by chance alone, fail to include the true mean between our limits. For example, if $\alpha=0.05$, we can expect 5 of 100 confidence intervals to fail to include the true mean.

To compute the limits, the sample mean, $\overline{X}$,; the standard error, s_x; and the degrees of freedom, n-1; must be known. A $t_{\alpha/2,n-1}$ value from tables of Student's t is obtained corresponding to n-1 degrees of freedom and probability α. The computation is:

$$CL_1 = \overline{X} - (t_{\alpha/2}) \cdot (s_{\overline{X}})$$
$$CL_2 = \overline{X} + (t_{\alpha/2}) \cdot (s_{\overline{X}})$$

7.6.6 Validating Normality and Homogeneity of Variance Assumptions[1]

7.6.6.1 Introduction

The t-test and the analysis of variance are parametric procedures based on the assumptions that the observations within treatments are independent and normally distributed, and that the variance of the observations is homogeneous across all groups of observations. These assumptions should be checked prior to using these tests, to determine if they have been met. Tests for validating the assumptions are provided in the following discussion. If the tests fail (if the data do not meet the assumptions), a non-parametric procedure such as Friedman's Test or Wilcoxon's Rank Sum Test may be more appropriate. However, the decision on whether to use parametric or non-parametric tests may be a judgment call, and a statistician should be consulted in selecting the analysis.

7.6.6.2 Test for Normal Distribution of Data

A formal test for normality is the Shapiro-Wilk's Test. The test statistic is obtained by dividing the square of an appropriate linear combination of the sample order statistics by the usual symmetric estimate of variance. The calculated W must be greater than zero and less than or equal to one. This test is recommended for a sample size of 50 or less. If the sample size is greater than 50, the Kolomogorov "D" statistic is recommended. An example of the Shapiro-Wilk's test is provided below.

The example uses macroinvertebrate biomass data. The same data are used in the discussion of the homogeneity of variance determination and the one-way analysis of variance example. The data and the mean and standard deviation of the observations at each time are listed in Table 17.

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[1] Adapted and modified from USEPA, 1989

The first step of the test for normality is to center the observations by subtracting the mean of all the observations within a concentration from each observation in that concentration. The centered observations are listed in Table 18.

Calculate the denominator, D, of the test statistic:

$$D = \sum_{i} X_i^2 - \frac{(\sum_{i} X_i)^2}{n}$$

$$D = 2,664,705 - \frac{(-3)^2}{12} = 2,664,704$$

Where: X_i = The i^{th} centered observations.

n = The total number of observations.

Order the centered observations from smallest to largest.

$$X^{(1)} - X^{(2)} \quad . \quad . \quad . \quad - \quad X^{(n)}$$

Where $X^{(i)}$ denotes the ith ordered observation. The ordered observations are listed in Table 19.

From Table 21, for the number of observations, n, obtain the coefficients a_1, a_2, ..., a_k, where k is approximately n/2. For the data in this example, n = 12, k = 6. The a_i values are listed in Table 20.

Compute the test statistic, W, as follows:

$$W = \frac{1}{D} [\sum_{i=1}^{k} a_i (X^{(n-i+1)} - X^{(i)})]$$

$$W = \frac{1}{2,664,704} (1610)^2 = 0.973$$

The differences, $X^{(n-i+1)} - X^{(i)}$, are listed in Table 20.

The decision rule for this test is to compare the critical value from Table 22 to the computed W. If the computed value is less than the critical value, conclude that the data are not normally distributed. For this example, the critical value at a significance level of 0.01 and 12 observations (n) is 0.805. The calculated value, 0.973, is not less than the critical value. Thus, the conclusion of the test is that the data are normally distributed.

TABLE 17. MACROINVERTEBRATE BIOMASS COLLECTED AT DIFFERENT TIMES OF DAY FROM THE LITTLE MIAMI RIVER AT MILFORD, OHIO

Sampling Time	Replicate number	Biomass (mg dry wt.)	S^2	$\bar{X}$
9:00AM - 1:00PM	1	1678	80,161	1418
	2	1211		
	3	1644		
	4	1137		
1:00AM - 4:00PM	1	1604	209,392	1975
	2	1639		
	3	2077		
	4	2581		
4:00PM - 7:00PM	1	4276	598,680	3328
	2	2400		
	3	3183		
	4	3451		

TABLE 18. EXAMPLE OF SHAPIRO-WILK'S TEST: CENTERED OBSERVATIONS

Sampling Time	Replicate			
	1	2	3	4
9:00AM - 1:00PM	260	-207	226	-281
1:00PM - 4:00PM	-371	-336	102	606
4:00PM - 7:00PM	948	-928	-145	123

TABLE 19. EXAMPLE OF SHAPIRO-WILK'S TEST: ORDERED OBSERVATIONS

i	$X_{(i)}$	i	$X_{(i)}$
1	-928	7	102
2	-371	8	123
3	-336	9	226
4	-280	10	260
5	-207	11	606
6	-145	12	948

TABLE 20. EXAMPLE OF SHAPIRO-WILK'S TEST: TABLE OF COEFFICIENTS AND DIFFERENCES

i	a_i	$X^{(n-i-1)} - X^{(i)}$	
1	.5475	1876	$X^{(12)}-X^{(1)}$
2	.3325	977	$X^{(11)}-X^{(2)}$
3	.2347	596	$X^{(10)}-X^{(3)}$
4	.1586	507	$X^{(9)}-X^{(4)}$
5	.0922	330	$X^{(8)}-X^{(5)}$
6	.0303	247	$X^{(7)}-X^{(6)}$

TABLE 21. COEFFICIENTS FOR THE SHAPIRO-WILKS TEST[1]

i \ n	2	3	4	5	6	7	8	9	10
1	0.7071	0.7071	0.6872	0.6646	0.6431	0.6233	0.6052	0.5888	0.5739
2	—	0.0000	0.1667	0.2413	0.2806	0.3031	0.3164	0.3244	0.3291
3	—	—	—	0.0000	0.0875	0.1401	0.1743	0.1976	0.2141
4	—	—	—	—	—	0.0000	0.0561	0.0947	0.1224
5	—	—	—	—	—	—	—	0.0000	0.0399

i \ n	11	12	13	14	15	16	17	18	19	20
1	0.5601	0.5475	0.5359	0.5251	0.5150	0.5056	0.4968	0.4886	0.4808	0.4734
2	0.3315	0.3325	0.3325	0.3318	0.3306	0.3290	0.3273	0.3253	0.3232	0.3211
3	0.2260	0.2347	0.2412	0.2460	0.2495	0.2521	0.2540	0.2553	0.2561	0.2565
4	0.1429	0.1586	0.1707	0.1802	0.1878	0.1939	0.1988	0.2027	0.2059	0.2085
5	0.0695	0.0922	0.1099	0.1240	0.1353	0.1447	0.1524	0.1587	0.1641	0.1686
6	0.0000	0.0303	0.0539	0.0727	0.0880	0.1005	0.1109	0.1197	0.1271	0.1334
7	—	—	0.0000	0.0240	0.0433	0.0593	0.0725	0.0837	0.0932	0.1013
8	—	—	—	—	0.0000	0.0196	0.0359	0.0496	0.0612	0.0711
9	—	—	—	—	—	—	0.0000	0.0163	0.0303	0.0422
10	—	—	—	—	—	—	—	—	0.0000	0.0140

i \ n	21	22	23	24	25	26	27	28	29	30
1	0.4643	0.4590	0.4542	0.4493	0.4450	0.4407	0.4366	0.4328	0.4291	0.4254
2	0.3185	0.3156	0.3126	0.3098	0.3069	0.3043	0.3018	0.2992	0.2968	0.2944
3	0.2578	0.2571	0.2563	0.2554	0.2543	0.2533	0.2522	0.2510	0.2499	0.2487
4	0.2119	0.2131	0.2139	0.2145	0.2148	0.2151	0.2152	0.2151	0.2150	0.2148
5	0.1736	0.1764	0.1787	0.1807	0.1822	0.1836	0.1848	0.1857	0.1864	0.1870
6	0.1399	0.1443	0.1480	0.1512	0.1539	0.1563	0.1584	0.1601	0.1616	0.1630
7	0.1092	0.1150	0.1201	0.1245	0.1283	0.1316	0.1346	0.1372	0.1395	0.1415
8	0.0804	0.0878	0.0941	0.0997	0.1046	0.1089	0.1128	0.1162	0.1192	0.1219
9	0.0530	0.0618	0.0696	0.0764	0.0823	0.0876	0.0923	0.0965	0.1002	0.1036
10	0.0263	0.0368	0.0459	0.0539	0.0610	0.0672	0.0728	0.0778	0.0822	0.0862
11	0.0000	0.0122	0.0228	0.0321	0.0403	0.0476	0.0540	0.0598	0.0650	0.0697
12	—	—	0.0000	0.0107	0.0200	0.0284	0.0358	0.0424	0.0483	0.0537
13	—	—	—	—	0.0000	0.0094	0.0178	0.0253	0.0320	0.0381
14	—	—	—	—	—	—	0.0000	0.0084	0.0159	0.0227
15	—	—	—	—	—	—	—	—	0.0000	0.0076

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[1] Taken from Conover, 1980.

TABLE 21. COEFFICIENT FOR THE SHAPIRO-WILKS TEST (Continued)

n i	31	32	33	34	35	36	37	38	39	40
1	0.4220	0.4188	0.4156	0.4127	0.4096	0.4068	0.4040	0.4015	0.3989	0.3964
2	0.2921	0.2898	0.2876	0.2854	0.2834	0.2813	0.2794	0.2774	0.2755	0.2737
3	0.2475	0.2462	0.2451	0.2439	0.2427	0.2415	0.2403	0.2391	0.2380	0.2368
4	0.2145	0.2141	0.2137	0.2132	0.2127	0.2121	0.2116	0.2110	0.2104	0.2098
5	0.1874	0.1878	0.1880	0.1882	0.1883	0.1883	0.1883	0.1881	0.1880	0.1878
6	0.1641	0.1651	0.1660	0.1667	0.1673	0.1678	0.1683	0.1686	0.1689	0.1691
7	0.1433	0.1449	0.1463	0.1475	0.1487	0.1496	0.1505	0.1513	0.1520	0.1526
8	0.1243	0.1265	0.1284	0.1301	0.1317	0.1331	0.1344	0.1356	0.1366	0.1376
9	0.1066	0.1093	0.1118	0.1140	0.1160	0.1179	0.1196	0.1211	0.1225	0.1237
10	0.0899	0.0931	0.0961	0.0988	0.1013	0.1036	0.1056	0.1075	0.1092	0.1108
11	0.0739	0.0777	0.0812	0.0844	0.0873	0.0900	0.0924	0.0947	0.0967	0.0986
12	0.0585	0.0629	0.0669	0.0706	0.0739	0.0770	0.0798	0.0824	0.0848	0.0870
13	0.0435	0.0485	0.0530	0.0572	0.0610	0.0645	0.0677	0.0706	0.0733	0.0759
14	0.0289	0.0344	0.0395	0.0441	0.0484	0.0523	0.0559	0.0592	0.0622	0.0651
15	0.0144	0.0206	0.0262	0.0314	0.0361	0.0404	0.0444	0.0481	0.0515	0.0546
16	0.0000	0.0068	0.0131	0.0187	0.0239	0.0287	0.0331	0.0372	0.0409	0.0444
17	—	—	0.0000	0.0062	0.0119	0.0172	0.0220	0.0264	0.0305	0.0343
18	—	—	—	—	0.0000	0.0057	0.0110	0.0158	0.0203	0.0244
19	—	—	—	—	—	—	0.0000	0.0053	0.0101	0.0146
20	—	—	—	—	—	—	—	—	0.0000	0.0049

n i	41	42	43	44	45	46	47	48	49	50
1	0.3940	0.3917	0.3894	0.3872	0.3850	0.3830	0.3808	0.3789	0.3770	0.3751
2	0.2719	0.2701	0.2684	0.2667	0.2651	0.2635	0.2620	0.2604	0.2589	0.2574
3	0.2357	0.2345	0.2334	0.2323	0.2313	0.2302	0.2291	0.2281	0.2271	0.2260
4	0.2091	0.2085	0.2078	0.2072	0.2065	0.2058	0.2052	0.2045	0.2038	0.2032
5	0.1876	0.1874	0.1871	0.1868	0.1865	0.1862	0.1859	0.1855	0.1851	0.1847
6	0.1693	0.1694	0.1695	0.1695	0.1695	0.1695	0.1695	0.1693	0.1692	0.1691
7	0.1531	0.1535	0.1539	0.1542	0.1545	0.1548	0.1550	0.1551	0.1553	0.1554
8	0.1384	0.1392	0.1398	0.1405	0.1410	0.1415	0.1420	0.1423	0.1427	0.1430
9	0.1249	0.1259	0.1269	0.1278	0.1286	0.1293	0.1300	0.1306	0.1312	0.1317
10	0.1123	0.1136	0.1149	0.1160	0.1170	0.1180	0.1189	0.1197	0.1205	0.1212
11	0.1004	0.1020	0.1035	0.1049	0.1062	0.1073	0.1085	0.1095	0.1105	0.1113
12	0.0891	0.0909	0.0927	0.0943	0.0959	0.0972	0.0986	0.0998	0.1010	0.1020
13	0.0782	0.0804	0.0824	0.0842	0.0860	0.0876	0.0892	0.0906	0.0919	0.0932
14	0.0677	0.0701	0.0724	0.0745	0.0765	0.0783	0.0801	0.0817	0.0832	0.0846
15	0.0575	0.0602	0.0628	0.0651	0.0673	0.0694	0.0713	0.0731	0.0748	0.0764
16	0.0476	0.0506	0.0534	0.0560	0.0584	0.0607	0.0628	0.0648	0.0667	0.0685
17	0.0379	0.0411	0.0442	0.0471	0.0497	0.0522	0.0546	0.0568	0.0588	0.0608
18	0.0283	0.0318	0.0352	0.0383	0.0412	0.0439	0.0465	0.0489	0.0511	0.0532
19	0.0188	0.0227	0.0263	0.0296	0.0328	0.0357	0.0385	0.0411	0.0436	0.0459
20	0.0094	0.0136	0.0175	0.0211	0.0245	0.0277	0.0307	0.0335	0.0361	0.0386
21	0.0000	0.0045	0.0087	0.0126	0.0163	0.0197	0.0229	0.0259	0.0288	0.0314
22		—	0.0000	0.0042	0.0081	0.0118	0.0153	0.0185	0.0215	0.0244
23	—	—	—	—	0.0000	0.0039	0.0076	0.0111	0.0143	0.0174
24	—	—	—	—	—	—	0.0000	0.0037	0.0071	0.0104
25	—	—	—	—	—	—	—	—	0.0000	0.0035

TABLE 22. QUANTILES OF THE SHAPIRO-WILKS TEST STATISTIC[1]

n	*0.01*	*0.02*	*0.05*	*0.10*	*0.50*	*0.90*	*0.95*	*0.98*	*0.99*
3	0.753	0.756	0.767	0.789	0.959	0.998	0.999	1.000	1.000
4	0.687	0.707	0.748	0.792	0.935	0.987	0.992	0.996	0.997
5	0.686	0.715	0.762	0.806	0.927	0.979	0.986	0.991	0.993
6	0.713	0.743	0.788	0.826	0.927	0.974	0.981	0.986	0.989
7	0.730	0.760	0.803	0.838	0.928	0.972	0.979	0.985	0.988
8	0.749	0.778	0.818	0.851	0.932	0.972	0.978	0.984	0.987
9	0.764	0.791	0.829	0.859	0.935	0.972	0.978	0.984	0.986
10	0.781	0.806	0.842	0.869	0.938	0.972	0.978	0.983	0.986
11	0.792	0.817	0.850	0.876	0.940	0.973	0.979	0.984	0.986
12	0.805	0.828	0.859	0.883	0.943	0.973	0.979	0.984	0.986
13	0.814	0.837	0.866	0.889	0.945	0.974	0.979	0.984	0.986
14	0.825	0.846	0.874	0.895	0.947	0.975	0.980	0.984	0.986
15	0.835	0.855	0.881	0.901	0.950	0.975	0.980	0.984	0.987
16	0.844	0.863	0.887	0.906	0.952	0.976	0.981	0.985	0.987
17	0.851	0.869	0.892	0.910	0.954	0.977	0.981	0.985	0.987
18	0.858	0.874	0.897	0.914	0.956	0.978	0.982	0.986	0.988
19	0.863	0.879	0.901	0.917	0.957	0.978	0.982	0.986	0.988
20	0.868	0.884	0.905	0.920	0.959	0.979	0.983	0.986	0.988
21	0.873	0.888	0.908	0.923	0.960	0.980	0.983	0.987	0.989
22	0.878	0.892	0.911	0.926	0.961	0.980	0.984	0.987	0.989
23	0.881	0.895	0.914	0.928	0.962	0.981	0.984	0.987	0.989
24	0.884	0.898	0.916	0.930	0.963	0.981	0.984	0.987	0.989
25	0.888	0.901	0.918	0.931	0.964	0.981	0.985	0.988	0.989
26	0.891	0.904	0.920	0.933	0.965	0.982	0.985	0.988	0.989
27	0.894	0.906	0.923	0.935	0.965	0.982	0.985	0.988	0.990
28	0.896	0.908	0.924	0.936	0.966	0.982	0.985	0.988	0.990
29	0.898	0.910	0.926	0.937	0.966	0.982	0.985	0.988	0.990
30	0.900	0.912	0.927	0.939	0.967	0.983	0.985	0.988	0.990
31	0.902	0.914	0.929	0.940	0.967	0.983	0.986	0.988	0.990
32	0.904	0.915	0.930	0.941	0.968	0.983	0.986	0.988	0.990
33	0.906	0.917	0.931	0.942	0.968	0.983	0.986	0.989	0.990
34	0.908	0.919	0.933	0.943	0.969	0.983	0.986	0.989	0.990
35	0.910	0.920	0.934	0.944	0.969	0.984	0.986	0.989	0.990
36	0.912	0.922	0.935	0.945	0.970	0.984	0.986	0.989	0.990
37	0.914	0.924	0.936	0.946	0.970	0.984	0.987	0.989	0.990
38	0.916	0.925	0.938	0.947	0.971	0.984	0.987	0.989	0.990
39	0.917	0.927	0.939	0.948	0.971	0.984	0.987	0.989	0.991
40	0.919	0.928	0.940	0.949	0.972	0.985	0.987	0.989	0.991
41	0.920	0.929	0.941	0.950	0.972	0.985	0.987	0.989	0.991
42	0.922	0.930	0.942	0.951	0.972	0.985	0.987	0.989	0.991
43	0.923	0.932	0.943	0.951	0.973	0.985	0.987	0.990	0.991
44	0.924	0.933	0.944	0.952	0.973	0.985	0.987	0.990	0.991
45	0.926	0.934	0.945	0.953	0.973	0.985	0.988	0.990	0.991
46	0.927	0.935	0.945	0.953	0.974	0.985	0.988	0.990	0.991
47	0.928	0.936	0.946	0.954	0.974	0.985	0.988	0.990	0.991
48	0.929	0.937	0.947	0.954	0.974	0.985	0.988	0.990	0.991
49	0.929	0.937	0.947	0.955	0.974	0.985	0.988	0.990	0.991
50	0.930	0.938	0.947	0.955	0.974	0.985	0.988	0.990	0.991

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[1] Taken from Conover, 1980.

7.6.6.3 Test for Homogeneity of Variance

For the analysis of variance, the variances of the data obtained for each group of observations are assumed to be equal. Bartlett's Test is a formal test of this assumption. In using this test, it is assumed that the data are normally distributed.

The data used in this example are biomass data from the one-way analysis of variance example and the Shapiro-Wilk's Test example. These data are listed in Table 17, together with the calculated sample variance for each group of observations.

The test statistic for Bartlett's Test (Snedecor and Cochran, 1980) is as follows:

$$B = \frac{[(\sum_{i=1}^{p} V_i)\ln \bar{S}^2 - \sum_{i=1}^{p} (V_i \ln S_i^2)]}{C}$$

Where: V_i = Degrees of freedom for each time
p = Number of levels of times

$\bar{S}^2$ = The average of the individual variances.
ln = Log_e

$$C = 1 + [\frac{1}{3(p-1)}][\sum_{i=1}^{p} \frac{1}{V_i} - \frac{1}{\sum_{i=1}^{p} V_i}]$$

Since B is approximately distributed as chi-square with p - 1 degrees of freedom when the variances are equal, the appropriate critical value is obtained from a table of the chi-square distribution for p - 1 degrees of freedom and a significance level of α. If B is less than the critical value then the variances are assumed to be equal.

For the data in this example, $V_i = 4 - 1 = 3$, $p = 3$, $\bar{S}^2 = 296{,}078$, and $C = 1.148$. The calculated value is:

$$B = \frac{[(\sum_{i=1}^{3} 3)\ln \bar{S}^2 - 3\sum_{i=1}^{3} (\ln S_i^2)]}{1.148}$$

$$B = \frac{9(12.598) - 3(36.846)}{1.148} = 2.477$$

Since B is approximately distributed as chi-square with 2 degrees of freedom when the variances are equal, the appropriate critical value for the test is 9.210 (see a χ^2 table) for a significance level of 0.01. Since B = 2.477 is less than the critical value of 9.210, conclude that the variances are not different.

7.6.6.4 Transformations of the Data

When the assumptions of normality and/or homogeneity of variance are not met, transformations of the data may remedy the problem, so that the data can be analyzed by parametric procedures, rather than a non-parametric technique such as Friedman's Test or Wilcoxon's Rank Sum Test. Examples of transformations include log, square root, arc sine square root, and reciprocals. After the data have been transformed, Shapiro-Wilk's and Bartlett's test should be performed on the transformed observations to determine whether the assumptions of normality and/or homogeneity of variance are met.

Table 23 is reproduced here with permission from Lloyd, Zar, and Karr (1968) for use in calculating mean diversity ($\bar{d}$) (see 7.3.10, page 114). To use the table, find the number of individuals (n) in column 1 and read the log of that number in column 3 (n log n).

TABLE 23. FUNCTIONS FOR CALCULATING SPECIES DIVERSITY AND (FOR PERFECTLY RANDOM SAMPLING) ITS STANDARD ERROR LOGARITHMS ARE TO BASE 10. TABLE VALUES ARE ACCURATE TO WITHIN ±1 IN THE EIGHTH SIGNIFICANT FIGURE.

n	log n!	n log n	n log^2 n
1	.0000	.0000	.0000
2	.3010	.6021	.1812
3	.7782	1.4314	.6829
4	1.3802	2.4082	1.4499
5	2.0792	3.4949	2.4428
6	2.8573	4.6689	3.6331
7	3.7024	5.9157	4.9993
8	4.6055	7.2247	6.5246
9	5.5598	8.5882	8.1952
10	6.5598	10.0000	10.0000
11	7.6012	11.4553	11.9295
12	8.6803	12.9502	13.9756
13	9.7943	14.4813	16.1313
14	10.9404	16.0458	18.3905
15	12.1165	17.6414	20.7479
16	13.3206	19.2659	23.1985
17	14.5511	20.9176	25.7381
18	15.8063	22.5949	28.3628
19	17.0851	24.2963	31.0690
20	18.3861	26.0206	33.8536
21	19.7083	27.7666	36.7135
22	21.0508	29.5333	39.6462
23	22.4125	31.3197	42.6490
24	23.7927	33.1251	45.7196
25	25.1906	34.9485	48.8559
26	26.6056	36.7893	52.0559
27	28.0370	38.6468	55.3177
28	29.4841	40.5204	58.6395
29	30.9463	42.4095	62.0196
30	32.4237	44.3136	65.4566
31	33.9150	46.2322	68.9490
32	35.4202	48.1648	72.4952
33	36.9387	50.1110	76.0942
34	38.4702	52.0703	79.7445
35	40.0142	54.0424	83.4451
36	41.5705	56.0269	87.1948
37	43.1387	58.0235	90.9925
38	44.7185	60.0318	94.8372
39	46.3096	62.0515	98.7280
40	47.9116	64.0824	102.6638
41	49.5244	66.1241	106.6439
42	51.1477	68.1765	110.6674
43	52.7811	70.2391	114.7334
44	54.4246	72.3119	118.8412
45	56.0778	74.3946	122.9900
46	57.7406	76.4869	127.1791
47	59.4127	78.5886	131.4078
48	61.0939	80.6996	135.6755
49	62.7841	82.8196	139.9814
50	64.4831	84.9485	144.3250
51	66.1906	87.0861	148.7056
52	67.9066	89.2322	153.1227
53	69.6309	91.3866	157.5757
54	71.3633	93.5493	162.0642
55	73.1037	95.7199	166.5874
56	74.8519	97.8985	171.1450
57	76.6077	100.0849	175.7365
58	78.3712	102.2788	180.3613
59	80.1420	104.4803	185.0191
60	81.9202	106.6891	189.7093
61	83.7055	108.9051	194.4316
62	85.4979	111.1283	199.1854
63	87.2972	113.3585	203.9705
64	89.1034	115.5955	208.7863
65	90.9163	117.8394	213.6326
66	92.7359	120.0899	218.5088
67	94.5619	122.3470	223.4148
68	96.3945	124.6106	228.3500
69	98.2333	126.8806	233.3143
70	100.0784	129.1569	238.3071
71	101.9297	131.4393	243.3282
72	103.7870	133.7279	248.3772
73	105.6503	136.0226	253.4540
74	107.5196	138.3231	258.5580
75	109.3946	140.6296	263.6891
76	111.2754	142.9418	268.8469
77	113.1619	145.2598	274.0312
78	115.0540	147.5834	279.2417
79	116.9516	149.9125	284.4781
80	118.8547	152.2472	289.7401
81	120.7632	154.5873	295.0275
82	122.6770	156.9327	300.3400
83	124.5961	159.2835	305.6774
84	126.5204	161.6395	311.0395
85	128.4498	164.0006	316.4259
86	130.3843	166.3669	321.8364
87	132.3238	168.7382	327.2709
88	134.2683	171.1145	332.7291
89	136.2177	173.4957	338.2108
90	138.1719	175.8818	343.7157
91	140.1310	178.2728	349.2437
92	142.0948	180.6685	354.7946
93	144.0632	183.0689	360.3680
94	146.0364	185.4740	365.9640
95	148.0141	187.8837	371.5821
96	149.9964	190.2980	377.2223
97	151.9831	192.7169	382.8844
98	153.9744	195.1402	388.5682
99	155.9700	197.5679	394.2734
100	157.9700	200.0000	400.0000
101	159.9743	202.4365	405.7477
102	161.9829	204.8772	411.5164
103	163.9958	207.3222	417.3059
104	166.0128	209.7715	423.1160
105	168.0340	212.2249	428.9466
106	170.0593	214.6824	434.7976
107	172.0887	217.1441	440.6686
108	174.1221	219.6098	446.5597
109	176.1595	222.0795	452.4706
110	178.2009	224.5532	458.4013
111	180.2462	227.0309	464.3514
112	182.2955	229.5124	470.3210
113	184.3485	231.9979	476.3098
114	186.4054	234.4872	482.3178
115	188.4661	236.9803	488.3447
116	190.5306	239.4771	494.3905
117	192.5988	241.9777	500.4550
118	194.6707	244.4821	506.5380
119	196.7462	246.9901	512.6395
120	198.8254	249.5017	518.7594
121	200.9082	252.0170	524.8974
122	202.9945	254.5359	531.0535
123	205.0844	257.0583	537.2275
124	207.1779	259.5843	543.4194
125	209.2748	262.1138	549.6290
126	211.3751	264.6467	555.8561
127	213.4790	267.1831	562.1007
128	215.5862	269.7229	568.3627
129	217.6967	272.2661	574.6420
130	219.8107	274.8126	580.9383
131	221.9280	277.3625	587.2517
132	224.0485	279.9158	593.5821
133	226.1724	282.4723	599.9292
134	228.2995	285.0320	606.2930
135	230.4298	287.5951	612.6735
136	232.5634	290.1613	619.0704
137	234.7001	292.7307	625.4837
138	236.8400	295.3033	631.9134
139	238.9830	297.8791	638.3592
140	241.1291	300.4579	644.8212

TABLE 23. (Continued)

n	log n!	n log n	$n \log^2 n$
141	243.2783	303.0399	651.2991
142	245.4306	305.6249	657.7930
143	247.5860	308.2131	664.3027
144	249.7443	310.8042	670.8281
145	251.9057	313.3984	677.3692
146	254.0700	315.9955	683.9258
147	256.2374	318.5956	690.4979
148	258.4076	321.1987	697.0853
149	260.5808	323.8048	703.6880
150	262.7569	326.4137	710.3060
151	264.9359	329.0255	716.9390
152	267.1177	331.6402	723.5871
153	269.3024	334.2578	730.2501
154	271.4899	336.8782	736.9280
155	273.6803	339.5014	743.6207
156	275.8734	342.1274	750.3281
157	278.0693	344.7562	757.0501
158	280.2679	347.3878	763.7867
159	282.4693	350.0221	770.5377
160	284.6735	352.6592	777.3032
161	286.8803	355.2990	784.0830
162	289.0898	357.9414	790.8770
163	291.3020	360.5866	797.6852
164	293.5168	363.2344	804.5075
165	295.7343	365.8849	811.3438
166	297.9544	368.5379	818.1941
167	300.1771	371.1936	825.0582
168	302.4024	373.8520	831.9362
169	304.6303	376.5129	838.8280
170	306.8608	379.1763	845.7334
171	309.0938	381.8423	852.6524
172	311.3293	384.5109	859.5850
173	313.5674	387.1820	866.5311
174	315.8079	389.8556	873.4906
175	318.0509	392.5317	880.4634
176	320.2965	395.2102	887.4496
177	322.5444	397.8913	894.4489
178	324.7948	400.5748	901.4615
179	327.0477	403.2607	908.4871
180	329.3030	405.9491	915.5257
181	331.5606	408.6398	922.5774
182	333.8207	411.3330	929.6419
183	336.0832	414.0285	936.7193
184	338.3480	416.7265	943.8096
185	340.6152	419.4268	950.9125
186	342.8847	422.1294	958.0282
187	345.1565	424.8344	965.1564
188	347.4307	427.5417	972.2973
189	349.7071	430.2513	979.4506
190	351.9859	432.9632	986.6164
191	354.2669	435.6774	993.7946
192	356.5502	438.3938	1000.9852
193	358.8358	441.1126	1008.1880
194	361.1236	443.8335	1015.4031
195	363.4136	446.5567	1022.6304
196	365.7059	449.2822	1029.8698
197	368.0003	452.0098	1037.1213
198	370.2970	454.7397	1044.3849
199	372.5959	457.4718	1051.6604
200	374.8969	460.2060	1058.9478
201	377.2001	462.9424	1066.2471
202	379.5054	465.6810	1073.5583
203	381.8129	468.4217	1080.8812
204	384.1226	471.1646	1088.2159
205	386.4343	473.9095	1095.5622
206	388.7482	476.6566	1102.9202
207	391.0642	479.4059	1110.2897
208	393.3822	482.1572	1117.6709
209	395.7024	484.9106	1125.0635
210	398.0246	487.6661	1132.4675
211	400.3489	490.4236	1139.8829
212	402.6752	493.1832	1147.3098
213	405.0036	495.9449	1154.7479
214	407.3340	498.7085	1162.1973
215	409.6664	501.4743	1169.6579
216	412.0009	504.2420	1177.1296
217	414.3373	507.0118	1184.6126
218	416.6758	509.7835	1192.1066
219	419.0162	512.5573	1199.6116
220	421.3587	515.3330	1207.1277
221	423.7031	518.1107	1214.6547
222	426.0494	520.8904	1222.1926
223	428.3977	523.6720	1229.7415
224	430.7480	526.4556	1237.3011
225	433.1002	529.2411	1244.8716
226	435.4543	532.0285	1252.4528
227	437.8103	534.8179	1260.0447
228	440.1682	537.6091	1267.6473
229	442.5281	540.4023	1275.2606
230	444.8898	543.1974	1282.8844
231	447.2534	545.9944	1290.5188
232	449.6189	548.7932	1298.1637
233	451.9862	551.5939	1305.8192
234	454.3555	554.3965	1313.4850
235	456.7265	557.2009	1321.1613
236	459.0994	560.0072	1328.8479
237	461.4742	562.8154	1336.5448
238	463.8508	565.6253	1344.2521
239	466.2292	568.4371	1351.9696
240	468.6094	571.2507	1359.6973
241	470.9914	574.0661	1367.4352
242	473.3752	576.8833	1375.1833
243	475.7608	579.7023	1382.9415
244	478.1482	582.5231	1390.7098
245	480.5374	585.3457	1398.4881
246	482.9283	588.1700	1406.2764
247	485.3210	590.9961	1414.0747
248	487.7154	593.8240	1421.8829
249	490.1116	596.6536	1429.7010
250	492.5096	599.4850	1437.5291
251	494.9093	602.3181	1445.3669
252	497.3107	605.1529	1453.2146
253	499.7138	607.9895	1461.0720
254	502.1186	610.8278	1468.9392
255	504.5252	613.6677	1476.8161
256	506.9334	616.5094	1484.7026
257	509.3433	619.3528	1492.5988
258	511.7549	622.1979	1500.5047
259	514.1682	625.0446	1508.4201
260	516.5832	627.8931	1516.3450
261	518.9999	630.7432	1524.2795
262	521.4182	633.5949	1532.2234
263	523.8381	636.4484	1540.1769
264	526.2597	639.3034	1548.1397
265	528.6830	642.1602	1556.1119
266	531.1078	645.0185	1564.0936
267	533.5344	647.8785	1572.0845
268	535.9625	650.7401	1580.0847
269	538.3922	653.6034	1588.0943
270	540.8236	656.4682	1596.1130
271	543.2566	659.3347	1604.1410
272	545.6912	662.2027	1612.1782
273	548.1273	665.0724	1620.2245
274	550.5651	667.9437	1628.2800
275	553.0044	670.8165	1636.3446
276	555.4453	673.6909	1644.4182
277	557.8878	676.5669	1652.5009
278	560.3318	679.4445	1660.5927
279	562.7774	682.3236	1668.6934
280	565.2246	685.2042	1676.8031
281	567.6733	688.0865	1684.9217
282	570.1235	690.9702	1693.0492
283	572.5753	693.8556	1701.1856
284	575.0287	696.7424	1709.3309
285	577.4835	699.6308	1717.4850
286	579.9399	702.5207	1725.6479
287	582.3977	705.4121	1733.8196
288	584.8571	708.3050	1742.0001
289	587.3180	711.1995	1750.1893
290	589.7804	714.0954	1758.3871
291	592.2443	716.9929	1766.5937
292	594.7097	719.8918	1774.8089
293	597.1766	722.7922	1783.0327
294	599.6449	725.6941	1791.2651
295	602.1147	728.5975	1799.5061
296	604.5860	731.5023	1807.7557
297	607.0588	734.4087	1816.0138
298	609.5330	737.3164	1824.2803
299	612.0087	740.2257	1832.5554
300	614.4858	743.1364	1840.8389
301	616.9644	746.0485	1849.1308
302	619.4444	748.9621	1857.4312
303	621.9258	751.8771	1865.7399
304	624.4087	754.7936	1874.0570
305	626.8930	757.7115	1882.3824
306	629.3787	760.6308	1890.7162
307	631.8659	763.5515	1899.0582
308	634.3544	766.4736	1907.4085
309	636.8444	769.3972	1915.7670
310	639.3357	772.3221	1924.1337
311	641.8285	775.2485	1932.5087
312	644.3226	778.1762	1940.8918
313	646.8182	781.1054	1949.2831
314	649.3151	784.0359	1957.6825
315	651.8134	786.9678	1966.0900
316	654.3131	789.9011	1974.5056
317	656.8142	792.8358	1982.9293
318	659.3166	795.7718	1991.3610
319	661.8204	798.7092	1999.8007
320	664.3255	801.6480	2008.2484
321	666.8320	804.5881	2016.7041
322	669.3399	807.5296	2025.1678
323	671.8491	810.4724	2033.6394
324	674.3596	813.4166	2042.1189
325	676.8715	816.3621	2050.6064
326	679.3847	819.3089	2059.1016
327	681.8993	822.2571	2067.6048
328	684.4152	825.2066	2076.1157
329	686.9324	828.1575	2084.6345
330	689.4509	831.1096	2093.1611
331	691.9707	834.0631	2101.6954
332	694.4918	837.0178	2110.2375
333	697.0143	839.9739	2118.7874
334	699.5380	842.9313	2127.3449
335	702.0631	845.8900	2135.9102
336	704.5894	848.8500	2144.4831
337	707.1170	851.8113	2153.0636
338	709.6460	854.7738	2161.6518
339	712.1762	857.7377	2170.2477
340	714.7076	860.7028	2178.8510
341	717.2404	863.6692	2187.4620
342	719.7744	866.6369	2196.0806
343	722.3097	869.6059	2204.7067
344	724.8463	872.5761	2213.3403
345	727.3841	875.5476	2221.9814
346	729.9232	878.5203	2230.6299
347	732.4635	881.4943	2239.2860
348	735.0051	884.4696	2247.9495
349	737.5479	887.4461	2256.6204
350	740.0920	890.4238	2265.2988
351	742.6373	893.4028	2273.9845
352	745.1838	896.3830	2282.6776
353	747.7316	899.3645	2291.3780
354	750.2806	902.3472	2300.0858
355	752.8308	905.3311	2308.8009
356	755.3823	908.3162	2317.5233
357	757.9349	911.3026	2326.2531
358	760.4888	914.2901	2334.9900
359	763.0439	917.2789	2343.7342
360	765.6002	920.2689	2352.4857
361	768.1577	923.2601	2361.2444
362	770.7164	926.2525	2370.0102
363	773.2764	929.2461	2378.7832
364	775.8375	932.2409	2387.5634
365	778.3997	935.2369	2396.3508
366	780.9632	938.2341	2405.1453
367	783.5279	941.2324	2413.9469
368	786.0937	944.2320	2422.7556

TABLE 23. (Continued)

n	log n!	n log n	n log² n
369	788.6608	947.2327	2431.5714
370	791.2290	950.2346	2440.3942
371	793.7983	953.2377	2449.2241
372	796.3689	956.2420	2458.0610
373	798.9406	959.2474	2466.9050
374	801.5135	962.2540	2475.7559
375	804.0875	965.2617	2484.6139
376	806.6627	968.2706	2493.4787
377	809.2390	971.2807	2502.3506
378	811.8165	974.2919	2511.2294
379	814.3952	977.3043	2520.1151
380	816.9749	980.3178	2529.0077
381	819.5559	983.3324	2537.9072
382	822.1379	986.3482	2546.8135
383	824.7211	989.3651	2555.7268
384	827.3055	992.3832	2564.6469
385	829.8909	995.4024	2573.5738
386	832.4775	998.4227	2582.5075
387	835.0652	1001.4441	2591.4480
388	837.6540	1004.4667	2600.3953
389	840.2440	1007.4904	2609.3493
390	842.8351	1010.5152	2618.3102
391	845.4272	1013.5411	2627.2777
392	848.0205	1016.5681	2636.2520
393	850.6149	1019.5963	2645.2330
394	853.2104	1022.6255	2654.2206
395	855.8070	1025.6558	2663.2150
396	858.4047	1028.6873	2672.2160
397	861.0035	1031.7198	2681.2237
398	863.6034	1034.7534	2690.2380
399	866.2044	1037.7882	2699.2589
400	868.8064	1040.8240	2708.2865
401	871.4096	1043.8609	2717.3206
402	874.0138	1046.8989	2726.3613
403	876.6191	1049.9379	2735.4086
404	879.2255	1052.9781	2744.4624
405	881.8329	1056.0193	2753.5228
406	884.4415	1059.0616	2762.5897
407	887.0510	1062.1049	2771.6631
408	889.6617	1065.1493	2780.7430
409	892.2734	1068.1948	2789.8293
410	894.8862	1071.2414	2798.9222
411	897.5001	1074.2890	2808.0215
412	900.1150	1077.3376	2817.1272
413	902.7309	1080.3874	2826.2394
414	905.3479	1083.4381	2835.3580
415	907.9660	1086.4900	2844.4830
416	910.5850	1089.5428	2853.6143
417	913.2052	1092.5967	2862.7521
418	915.8264	1095.6517	2871.8962
419	918.4486	1098.7077	2881.0467
420	921.0718	1101.7647	2890.2035
421	923.6961	1104.8228	2899.3666
422	926.3214	1107.8819	2908.5360
423	928.9478	1110.9420	2917.7117
424	931.5751	1114.0031	2926.8938
425	934.2035	1117.0653	2936.0820
426	936.8329	1120.1285	2945.2766
427	939.4633	1123.1927	2954.4774
428	942.0948	1126.2579	2963.6844
429	944.7272	1129.3242	2972.8976
430	947.3607	1132.3914	2982.1171
431	949.9952	1135.4597	2991.3428
432	952.6307	1138.5290	3000.5746
433	955.2672	1141.5993	3009.8126
434	957.9047	1144.6705	3019.0568
435	960.5431	1147.7428	3028.3071
436	963.1826	1150.8161	3037.5636
437	965.8231	1153.8904	3046.8261
438	968.4646	1156.9657	3056.0948
439	971.1071	1160.0419	3065.3696
440	973.7505	1163.1192	3074.6505
441	976.3949	1166.1974	3083.9374
442	979.0404	1169.2766	3093.2305
443	981.6868	1172.3568	3102.5295
444	984.3342	1175.4380	3111.8346
445	986.9825	1178.5202	3121.1458
446	989.6318	1181.6033	3130.4629
447	992.2822	1184.6875	3139.7861
448	994.9334	1187.7725	3149.1152
449	997.5857	1190.8586	3158.4504
450	1000.2389	1193.9456	3167.7915
451	1002.8931	1197.0336	3177.1385
452	1005.5482	1200.1226	3186.4915
453	1008.2043	1203.2125	3195.8505
454	1010.8614	1206.3033	3205.2154
455	1013.5194	1209.3952	3214.5862
456	1016.1783	1212.4880	3223.9629
457	1018.8382	1215.5817	3233.3455
458	1021.4991	1218.6764	3242.7339
459	1024.1609	1221.7720	3252.1283
460	1026.8237	1224.8686	3261.5284
461	1029.4874	1227.9661	3270.9345
462	1032.1520	1231.0646	3280.3464
463	1034.8176	1234.1640	3289.7641
464	1037.4841	1237.2643	3299.1876
465	1040.1516	1240.3656	3308.6169
466	1042.8200	1243.4678	3318.0521
467	1045.4893	1246.5710	3327.4930
468	1048.1595	1249.6750	3336.9396
469	1050.8307	1252.7801	3346.3921
470	1053.5028	1255.8860	3355.8503
471	1056.1758	1258.9928	3365.3142
472	1058.8498	1262.1006	3374.7838
473	1061.5246	1265.2093	3384.2592
474	1064.2004	1268.3189	3393.7403
475	1066.8771	1271.4295	3403.2271
476	1069.5547	1274.5409	3412.7196
477	1072.2332	1277.6533	3422.2177
478	1074.9126	1280.7665	3431.7216
479	1077.5930	1283.8807	3441.2310
480	1080.2742	1286.9958	3450.7462
481	1082.9564	1290.1118	3460.2669
482	1085.6394	1293.2287	3469.7933
483	1088.3234	1296.3465	3479.3253
484	1091.0082	1299.4651	3488.8630
485	1093.6940	1302.5847	3498.4062
486	1096.3806	1305.7052	3507.9550
487	1099.0681	1308.8266	3517.5094
488	1101.7565	1311.9489	3527.0693
489	1104.4458	1315.0720	3536.6349
490	1107.1360	1318.1961	3546.2059
491	1109.8271	1321.3210	3555.7825
492	1112.5191	1324.4468	3565.3646
493	1115.2119	1327.5735	3574.9523
494	1117.9057	1330.7011	3584.5454
495	1120.6003	1333.8296	3594.1441
496	1123.2957	1336.9589	3603.7482
497	1125.9921	1340.0891	3613.3578
498	1128.6893	1343.2202	3622.9730
499	1131.3874	1346.3522	3632.5935
500	1134.0864	1349.4850	3642.2195
501	1136.7862	1352.6187	3651.8510
502	1139.4869	1355.7533	3661.4879
503	1142.1885	1358.8887	3671.1302
504	1144.8909	1362.0250	3680.7779
505	1147.5942	1365.1621	3690.4310
506	1150.2984	1368.3002	3700.0896
507	1153.0034	1371.4390	3709.7535
508	1155.7093	1374.5788	3719.4228
509	1158.4160	1377.7193	3729.0974
510	1161.1235	1380.8608	3738.7775
511	1163.8320	1384.0031	3748.4629
512	1166.5412	1387.1462	3758.1536
513	1169.2514	1390.2902	3767.8496
514	1171.9623	1393.4350	3777.5510
515	1174.6741	1396.5807	3787.2577
516	1177.3868	1399.7272	3796.9697
517	1180.1003	1402.8746	3806.6870
518	1182.8146	1406.0228	3816.4095
519	1185.5298	1409.1718	3826.1374
520	1188.2458	1412.3217	3835.8705
521	1190.9626	1415.4724	3845.6089
522	1193.6803	1418.6240	3855.3526
523	1196.3988	1421.7764	3865.1015
524	1199.1181	1424.9296	3874.8556
525	1201.8383	1428.0836	3884.6150
526	1204.5592	1431.2385	3894.3795
527	1207.2811	1434.3942	3904.1493
528	1210.0037	1437.5507	3913.9243
529	1212.7271	1440.7080	3923.7045
530	1215.4514	1443.8662	3933.4899
531	1218.1765	1447.0252	3943.2804
532	1220.9024	145[illegible]850	3953.0761
533	1223.6292	1453.3[illegible]6	3962.8770
534	1226.3567	1456.5070	3972.6830
535	1229.0851	1459.6693	3982.4942
536	1231.8142	1462.8323	3992.3105
537	1234.5442	1465.9962	4002.1319
538	1237.2750	1469.1609	4011.9584
539	1240.0066	1472.3263	4021.7901
540	1242.7390	1475.4926	4031.6268
541	1245.4722	1478.6597	4041.4687
542	1248.2061	1481.8276	4051.3156
543	1250.9409	1484.9963	4061.1676
544	1253.6765	1488.1658	4071.0247
545	1256.4129	1491.3361	4080.8868
546	1259.1501	1494.5072	4090.7541
547	1261.8881	1497.6791	4100.6263
548	1264.6269	1500.8517	4110.5035
549	1267.3665	1504.0252	4120.3859
550	1270.1068	1507.1995	4130.2732
551	1272.8480	1510.3745	4140.1655
552	1275.5899	1513.5504	4150.0629
553	1278.3327	1516.7270	4159.9652
554	1281.0762	1519.9044	4169.8726
555	1283.8205	1523.0826	4179.7849
556	1286.5655	1526.2616	4189.7021
557	1289.3114	1529.4413	4199.6245
558	1292.0580	1532.6219	4209.5516
559	1294.8054	1535.8032	4219.4838
560	1297.5536	1538.9853	4229.4210
561	1300.3026	1542.1682	4239.3630
562	1303.0523	1545.3518	4249.3099
563	1305.8028	1548.5362	4259.2618
564	1308.5541	1551.7214	4269.2187
565	1311.3062	1554.9074	4279.1804
566	1314.0590	1558.0941	4289.1470
567	1316.8126	1561.2816	4299.1185
568	1319.5669	1564.4698	4309.0949
569	1322.3220	1567.6589	4319.0762
570	1325.0779	1570.8487	4329.0623
571	1327.8345	1574.0392	4339.0533
572	1330.5919	1577.2305	4349.0492
573	1333.3501	1580.4226	4359.0499
574	1336.1090	1583.6154	4369.0554
575	1338.8687	1586.8090	4379.0658
576	1341.6291	1590.0033	4389.0810
577	1344.3903	1593.1984	4399.1010
578	1347.1522	1596.3943	4409.1258
579	1349.9149	1599.5909	4419.1555
580	1352.6783	1602.7882	4429.1898
581	1355.4425	1605.9863	4439.2291
582	1358.2074	1609.1852	4449.2731
583	1360.9731	1612.3848	4459.3218
584	1363.7395	1615.5851	4469.3754
585	1366.5066	1618.7862	4479.4337
586	1369.2745	1621.9880	4489.4967
587	1372.0432	1625.1906	4499.5645
588	1374.8125	1628.3939	4509.6370
589	1377.5827	1631.5979	4519.7143
590	1380.3535	1634.8027	4529.7963
591	1383.1251	1638.0082	4539.8830
592	1385.8974	1641.2144	4549.9744
593	1388.6705	1644.4214	4560.0706
594	1391.4443	1647.6291	4570.1714
595	1394.2188	1650.8376	4580.2769
596	1396.9940	1654.0468	4590.3871

TABLE 23. (Continued)

n	log n!	n log n	n log² n
597	1399.7700	1657.2567	4600.5020
598	1402.5467	1660.4673	4610.6215
599	1405.3241	1663.6787	4620.7457
600	1408.1023	1666.8907	4630.8746
601	1410.8811	1670.1035	4641.0081
602	1413.6608	1673.3171	4651.1463
603	1416.4411	1676.5313	4661.2891
604	1419.2221	1679.7463	4671.4365
605	1422.0039	1682.9620	4681.5886
606	1424.7863	1686.1784	4691.7452
607	1427.5695	1689.3955	4701.9065
608	1430.3534	1692.6134	4712.0724
609	1433.1380	1695.8319	4722.2429
610	1435.9234	1699.0512	4732.4180
611	1438.7094	1702.2712	4742.5977
612	1441.4962	1705.4919	4752.7819
613	1444.2836	1708.7133	4762.9707
614	1447.0718	1711.9354	4773.1641
615	1449.8607	1715.1582	4783.3620
616	1452.6503	1718.3817	4793.5645
617	1455.4405	1721.6059	4803.7715
618	1458.2315	1724.8309	4813.9831
619	1461.0232	1728.0565	4824.1992
620	1463.8156	1731.2828	4834.4198
621	1466.6087	1734.5099	4844.6450
622	1469.4025	1737.7376	4854.8746
623	1472.1970	1740.9660	4865.1088
624	1474.9922	1744.1952	4875.3475
625	1477.7880	1747.4250	4885.5906
626	1480.5846	1750.6555	4895.8383
627	1483.3819	1753.8867	4906.0905
628	1486.1798	1757.1187	4916.3470
629	1488.9785	1760.3512	4926.6082
630	1491.7778	1763.5845	4936.8737
631	1494.5779	1766.8185	4947.1437
632	1497.3786	1770.0532	4957.4182
633	1500.1800	1773.2885	4967.6971
634	1502.9821	1776.5246	4977.9804
635	1505.7849	1779.7613	4988.2682
636	1508.5883	1782.9987	4998.5604
637	1511.3924	1786.2368	5008.8571
638	1514.1973	1789.4756	5019.1581
639	1517.0028	1792.7150	5029.4636
640	1519.8089	1795.9552	5039.7734
641	1522.6158	1799.1960	5050.0877
642	1525.4233	1802.4375	5060.4064
643	1528.2316	1805.6796	5070.7294
644	1531.0404	1808.9225	5081.0568
645	1533.8500	1812.1660	5091.3886
646	1536.6602	1815.4102	5101.7248
647	1539.4711	1818.6551	5112.0653
648	1542.2827	1821.9006	5122.4102
649	1545.0950	1825.1468	5132.7594
650	1547.9079	1828.3937	5143.1130
651	1550.7215	1831.6412	5153.4709
652	1553.5357	1834.8894	5163.8331
653	1556.3506	1838.1383	5174.1997
654	1559.1662	1841.3878	5184.5706
655	1561.9824	1844.6380	5194.9459
656	1564.7993	1847.8889	5205.3254
657	1567.6169	1851.1404	5215.7092
658	1570.4351	1854.3926	5226.0973
659	1573.2540	1857.6455	5236.4897
660	1576.0735	1860.8990	5246.8865
661	1578.8938	1864.1532	5257.2875
662	1581.7146	1867.4080	5267.6927
663	1584.5361	1870.6635	5278.1022
664	1587.3583	1873.9196	5288.5161
665	1590.1811	1877.1764	5298.9341
666	1593.0046	1880.4338	5309.3564
667	1595.8287	1883.6919	5319.7830
668	1598.6535	1886.9507	5330.2138
669	1601.4789	1890.2101	5340.6489
670	1604.3050	1893.4701	5351.0881
671	1607.1317	1896.7308	5361.5317
672	1609.9591	1899.9921	5371.9794
673	1612.7871	1903.2541	5382.4313
674	1615.6158	1906.5168	5392.8875
675	1618.4451	1909.7800	5403.3478
676	1621.2750	1913.0440	5413.8124
677	1624.1056	1916.3085	5424.2812
678	1626.9368	1919.5737	5434.7542
679	1629.7687	1922.8396	5445.2313
680	1632.6012	1926.1060	5455.7125
681	1635.4344	1929.3732	5466.1981
682	1638.2681	1932.6409	5476.6877
683	1641.1026	1935.9093	5487.1815
684	1643.9376	1939.1784	5497.6794
685	1646.7733	1942.4480	5508.1816
686	1649.6096	1945.7183	5518.6878
687	1652.4466	1948.9893	5529.1982
688	1655.2842	1952.2608	5539.7128
689	1658.1224	1955.5330	5550.2314
690	1660.9612	1958.8059	5560.7542
691	1663.8007	1962.0793	5571.2811
692	1666.6408	1965.3534	5581.8122
693	1669.4816	1968.6281	5592.3474
694	1672.3229	1971.9035	5602.8866
695	1675.1649	1975.1794	5613.4299
696	1678.0075	1978.4560	5623.9774
697	1680.8507	1981.7332	5634.5289
698	1683.6946	1985.0111	5645.0845
699	1686.5391	1988.2895	5655.6442
700	1689.3842	1991.5686	5666.2079
701	1692.2299	1994.8483	5676.7758
702	1695.0762	1998.1286	5687.3477
703	1697.9232	2001.4096	5697.9236
704	1700.7708	2004.6911	5708.5037
705	1703.6189	2007.9733	5719.0878
706	1706.4678	2011.2561	5729.6758
707	1709.3172	2014.5395	5740.2680
708	1712.1672	2017.8235	5750.8642
709	1715.0179	2021.1082	5761.4644
710	1717.8691	2024.3934	5772.0686
711	1720.7210	2027.6793	5782.6769
712	1723.5735	2030.9657	5793.2892
713	1726.4265	2034.2528	5803.9055
714	1729.2802	2037.5405	5814.5257
715	1732.1346	2040.8288	5825.1500
716	1734.9895	2044.1177	5835.7783
717	1737.8450	2047.4072	5846.4105
718	1740.7011	2050.6973	5857.0468
719	1743.5578	2053.9881	5867.6870
720	1746.4152	2057.2794	5878.3312
721	1749.2731	2060.5713	5888.9794
722	1752.1316	2063.8638	5899.6316
723	1754.9908	2067.1570	5910.2877
724	1757.8505	2070.4507	5920.9478
725	1760.7109	2073.7450	5931.6118
726	1763.5718	2077.0400	5942.2797
727	1766.4333	2080.3355	5952.9517
728	1769.2955	2083.6316	5963.6275
729	1772.1582	2086.9283	5974.3073
730	1775.0215	2090.2257	5984.9910
731	1777.8854	2093.5236	5995.6786
732	1780.7499	2096.8221	6006.3701
733	1783.6150	2100.1212	6017.0656
734	1786.4807	2103.4209	6027.7650
735	1789.3470	2106.7212	6038.4683
736	1792.2139	2110.0221	6049.1754
737	1795.0814	2113.3235	6059.8865
738	1797.9494	2116.6256	6070.6015
739	1800.8181	2119.9282	6081.3203
740	1803.6873	2123.2314	6092.0430
741	1806.5571	2126.5353	6102.7697
742	1809.4275	2129.8397	6113.5002
743	1812.2985	2133.1447	6124.2345
744	1815.1701	2136.4503	6134.9727
745	1818.0422	2139.7564	6145.7148
746	1820.9150	2143.0631	6156.4608
747	1823.7883	2146.3705	6167.2105
748	1826.6622	2149.6784	6177.9642
749	1829.5367	2152.9869	6188.7216
750	1832.4117	2156.2959	6199.4829
751	1835.2874	2159.6056	6210.2480
752	1838.1636	2162.9158	6221.0170
753	1841.0404	2166.2266	6231.7898
754	1843.9178	2169.5380	6242.5664
755	1846.7957	2172.8499	6253.3469
756	1849.6742	2176.1625	6264.1311
757	1852.5533	2179.4756	6274.9191
758	1855.4330	2182.7892	6285.7110
759	1858.3132	2186.1035	6296.5066
760	1861.1941	2189.4183	6307.3060
761	1864.0754	2192.7337	6318.1093
762	1866.9574	2196.0497	6328.9163
763	1869.8399	2199.3662	6339.7271
764	1872.7230	2202.6833	6350.5416
765	1875.6067	2206.0010	6361.3600
766	1878.4909	2209.3192	6372.1821
767	1881.3757	2212.6380	6383.0079
768	1884.2611	2215.9574	6393.8376
769	1887.1470	2219.2773	6404.6710
770	1890.0335	2222.5978	6415.5081
771	1892.9205	2225.9189	6426.3489
772	1895.8082	2229.2405	6437.1935
773	1898.6963	2232.5627	6448.0419
774	1901.5851	2235.8855	6458.8940
775	1904.4744	2239.2088	6469.7498
776	1907.3642	2242.5327	6480.6094
777	1910.2547	2245.8571	6491.4727
778	1913.1456	2249.1821	6502.3396
779	1916.0372	2252.5077	6513.2103
780	1918.9293	2255.8338	6524.0847
781	1921.8219	2259.1604	6534.9628
782	1924.7151	2262.4877	6545.8446
783	1927.6089	2265.8154	6556.7301
784	1930.5032	2269.1438	6567.6193
785	1933.3981	2272.4727	6578.5122
786	1936.2935	2275.8021	6589.4088
787	1939.1895	2279.1321	6600.3090
788	1942.0860	2282.4626	6611.2129
789	1944.9831	2285.7937	6622.1205
790	1947.8807	2289.1254	6633.0317
791	1950.7789	2292.4576	6643.9467
792	1953.6776	2295.7903	6654.8652
793	1956.5769	2299.1236	6665.7875
794	1959.4767	2302.4575	6676.7134
795	1962.3771	2305.7918	6687.6429
796	1965.2780	2309.1268	6698.5761
797	1968.1794	2312.4623	6709.5129
798	1971.0814	2315.7983	6720.4534
799	1973.9840	2319.1349	6731.3975
800	1976.8871	2322.4720	6742.3452
801	1979.7907	2325.8096	6753.2965
802	1982.6949	2329.1478	6764.2515
803	1985.5996	2332.4866	6775.2100
804	1988.5049	2335.8258	6786.1722
805	1991.4106	2339.1657	6797.1380
806	1994.3170	2342.5060	6808.1074
807	1997.2239	2345.8469	6819.0804
808	2000.1313	2349.1884	6830.0569
809	2003.0392	2352.5303	6841.0371
810	2005.9477	2355.8729	6852.0209
811	2008.8567	2359.2159	6863.0082
812	2011.7663	2362.5595	6873.9992
813	2014.6764	2365.9036	6884.9937
814	2017.5870	2369.2483	6895.9918
815	2020.4982	2372.5934	6906.9935
816	2023.4099	2375.9391	6917.9987
817	2026.3221	2379.2854	6929.0074
818	2029.2348	2382.6322	6940.0198
819	2032.1481	2385.9795	6951.0357
820	2035.0619	2389.3273	6962.0551
821	2037.9763	2392.6757	6973.0781
822	2040.8911	2396.0246	6984.1047
823	2043.8065	2399.3741	6995.1347
824	2046.7225	2402.7240	7006.1683

TABLE 23. (Continued)

n	log n!	n log n	n log^2 n
825	2049.6389	2406.0745	7017.2054
826	2052.5559	2409.4255	7028.2462
827	2055.4734	2412.7770	7039.2903
828	2058.3914	2416.1291	7050.3380
829	2061.3100	2419.4817	7061.3893
830	2064.2291	2422.8348	7072.4440
831	2067.1487	2426.1884	7083.5023
832	2070.0688	2429.5426	7094.5640
833	2072.9894	2432.8973	7105.6293
834	2075.9106	2436.2525	7116.6980
835	2078.8323	2439.6082	7127.7703
836	2081.7545	2442.9644	7138.8460
837	2084.6772	2446.3212	7149.9252
838	2087.6005	2449.6785	7161.0079
839	2090.5242	2453.0363	7172.0942
840	2093.4485	2456.3946	7183.1838
841	2096.3733	2459.7534	7194.2770
842	2099.2986	2463.1128	7205.3736
843	2102.2244	2466.4726	7216.4736
844	2105.1508	2469.8330	7227.5772
845	2108.0776	2473.1939	7238.6842
846	2111.0050	2476.5553	7249.7946
847	2113.9329	2479.9172	7260.9086
848	2116.8613	2483.2797	7272.0259
849	2119.7902	2486.6426	7283.1467
850	2122.7196	2490.0061	7294.2709
851	2125.6495	2493.3700	7305.3986
852	2128.5800	2496.7345	7316.5297
853	2131.5109	2500.0995	7327.6642
854	2134.4424	2503.4650	7338.8022
855	2137.3744	2506.8310	7349.9436
856	2140.3068	2510.1975	7361.0884
857	2143.2398	2513.5645	7372.2366
858	2146.1733	2516.9321	7383.3882
859	2149.1073	2520.3001	7394.5433
860	2152.0418	2523.6686	7405.7018
861	2154.9768	2527.0377	7416.8636
862	2157.9123	2530.4073	7428.0289
863	2160.8483	2533.7773	7439.1975
864	2163.7848	2537.1479	7450.3696
865	2166.7218	2540.5189	7461.5450
866	2169.6594	2543.8905	7472.7238
867	2172.5974	2547.2625	7483.9060
868	2175.5359	2550.6351	7495.0916
869	2178.4749	2554.0082	7506.2805
870	2181.4144	2557.3817	7517.4728
871	2184.3544	2560.7558	7528.6686
872	2187.2950	2564.1304	7539.8676
873	2190.2360	2567.5054	7551.0700
874	2193.1775	2570.8810	7562.2758
875	2196.1195	2574.2570	7573.4849
876	2199.0620	2577.6336	7584.6974
877	2202.0050	2581.0106	7595.9133
878	2204.9485	2584.3882	7607.1324
879	2207.8925	2587.7662	7618.3549
880	2210.8370	2591.1447	7629.5808
881	2213.7820	2594.5238	7640.8100
882	2216.7274	2597.9033	7652.0425
883	2219.6734	2601.2833	7663.2784
884	2222.6198	2604.6638	7674.5175
885	2225.5668	2608.0448	7685.7600
886	2228.5142	2611.4263	7697.0059
887	2231.4621	2614.8082	7708.2549
888	2234.4105	2618.1907	7719.5074
889	2237.3594	2621.5736	7730.7632
890	2240.3088	2624.9571	7742.0222
891	2243.2587	2628.3410	7753.2846
892	2246.2091	2631.7254	7764.5502
893	2249.1599	2635.1104	7775.8192
894	2252.1113	2638.4957	7787.0914
895	2255.0631	2641.8816	7798.3670
896	2258.0154	2645.2680	7809.6458
897	2260.9682	2648.6548	7820.9279
898	2263.9214	2652.0421	7832.2133
899	2266.8752	2655.4300	7843.5020
900	2269.8295	2658.8182	7854.7939
901	2272.7842	2662.2070	7866.0891
902	2275.7394	2665.5963	7877.3876
903	2278.6951	2668.9860	7888.6893
904	2281.6512	2672.3763	7899.9943
905	2284.6079	2675.7669	7911.3026
906	2287.5650	2679.1581	7922.6141
907	2290.5226	2682.5498	7933.9288
908	2293.4807	2685.9419	7945.2468
909	2296.4393	2689.3346	7956.5681
910	2299.3983	2692.7277	7967.8926
911	2302.3578	2696.1212	7979.2203
912	2305.3178	2699.5153	7990.5513
913	2308.2783	2702.9098	8001.8855
914	2311.2393	2706.3048	8013.2230
915	2314.2007	2709.7003	8024.5636
916	2317.1626	2713.0963	8035.9075
917	2320.1249	2716.4927	8047.2546
918	2323.0878	2719.8896	8058.6049
919	2326.0511	2723.2869	8069.9584
920	2329.0149	2726.6848	8081.3152
921	2331.9792	2730.0831	8092.6752
922	2334.9439	2733.4819	8104.0383
923	2337.9091	2736.8812	8115.4047
924	2340.8748	2740.2809	8126.7742
925	2343.8409	2743.6811	8138.1470
926	2346.8075	2747.0818	8149.5229
927	2349.7746	2750.4829	8160.9021
928	2352.7421	2753.8845	8172.2844
929	2355.7101	2757.2866	8183.6699
930	2358.6786	2760.6891	8195.0586
931	2361.6476	2764.0921	8206.4504
932	2364.6170	2767.4956	8217.8455
933	2367.5869	2770.8996	8229.2438
934	2370.5572	2774.3040	8240.6451
935	2373.5280	2777.7088	8252.0497
936	2376.4993	2781.1142	8263.4574
937	2379.4710	2784.5200	8274.8683
938	2382.4433	2787.9262	8286.2823
939	2385.4159	2791.3330	8297.6995
940	2388.3890	2794.7402	8309.1199
941	2391.3626	2798.1478	8320.5433
942	2394.3367	2801.5559	8331.9700
943	2397.3112	2804.9645	8343.3998
944	2400.2862	2808.3735	8354.8326
945	2403.2616	2811.7831	8366.2687
946	2406.2375	2815.1930	8377.7079
947	2409.2138	2818.6034	8389.1503
948	2412.1906	2822.0143	8400.5957
949	2415.1679	2825.4256	8412.0442
950	2418.1456	2828.8374	8423.4960
951	2421.1238	2832.2497	8434.9507
952	2424.1024	2835.6624	8446.4087
953	2427.0815	2839.0755	8457.8698
954	2430.0611	2842.4891	8469.3339
955	2433.0411	2845.9032	8480.8011
956	2436.0216	2849.3177	8492.2715
957	2439.0025	2852.7327	8503.7450
958	2441.9838	2856.1481	8515.2216
959	2444.9657	2859.5640	8526.7012
960	2447.9479	2862.9804	8538.1840
961	2450.9307	2866.3972	8549.6698
962	2453.9138	2869.8144	8561.1588
963	2456.8975	2873.2321	8572.6508
964	2459.8815	2876.6502	8584.1459
965	2462.8661	2880.0688	8595.6442
966	2465.8510	2883.4879	8607.1454
967	2468.8365	2886.9074	8618.6498
968	2471.8223	2890.3273	8630.1571
969	2474.8087	2893.7477	8641.6676
970	2477.7954	2897.1686	8653.1812
971	2480.7827	2900.5898	8664.6973
972	2483.7703	2904.0116	8676.2174
973	2486.7584	2907.4338	8687.7402
974	2489.7470	2910.8564	8699.2660
975	2492.7360	2914.2795	8710.7948
976	2495.7254	2917.7030	8722.3267
977	2498.7153	2921.1270	8733.8617
978	2501.7057	2924.5514	8745.3997
979	2504.6965	2927.9762	8756.9407
980	2507.6877	2931.4016	8768.4847
981	2510.6794	2934.8273	8780.0319
982	2513.6715	2938.2535	8791.5819
983	2516.6640	2941.6801	8803.1351
984	2519.6570	2945.1071	8814.6913
985	2522.6505	2948.5347	8826.2505
986	2525.6443	2951.9626	8837.8127
987	2528.6386	2955.3910	8849.3781
988	2531.6334	2958.8199	8860.9463
989	2534.6286	2962.2491	8872.5176
990	2537.6242	2965.6788	8884.0918
991	2540.6203	2969.1090	8895.6692
992	2543.6168	2972.5396	8907.2495
993	2546.6138	2975.9706	8918.8328
994	2549.6111	2979.4020	8930.4191
995	2552.6090	2982.8340	8942.0084
996	2555.6072	2986.2663	8953.6007
997	2558.6059	2989.6991	8965.1960
998	2561.6051	2993.1323	8976.7944
999	2564.6046	2996.5659	8988.3956
1000	2567.6046	3000.0000	9000.0000
1001	2570.6051	3003.4345	9011.6072
1002	2573.6059	3006.8694	9023.2174
1003	2576.6072	3010.3048	9034.8307
1004	2579.6090	3013.7406	9046.4469
1005	2582.6111	3017.1769	9058.0660
1006	2585.6137	3020.6136	9069.6881
1007	2588.6168	3024.0507	9081.3132
1008	2591.6202	3027.4882	9092.9413
1009	2594.6241	3030.9262	9104.5724
1010	2597.6284	3034.3646	9116.2063
1011	2600.6332	3037.8034	9127.8433
1012	2603.6384	3041.2427	9139.4832
1013	2606.6440	3044.6823	9151.1260
1014	2609.6500	3048.1225	9162.7719
1015	2612.6565	3051.5630	9174.4205
1016	2615.6634	3055.0040	9186.0723
1017	2618.6707	3058.4454	9197.7269
1018	2621.6784	3061.8872	9209.3845
1019	2624.6866	3065.3295	9221.0450
1020	2627.6952	3068.7722	9232.7084
1021	2630.7043	3072.2153	9244.3749
1022	2633.7137	3075.6588	9256.0441
1023	2636.7236	3079.1028	9267.7163
1024	2639.7339	3082.5471	9279.3915
1025	2642.7446	3085.9919	9291.0696
1026	2645.7557	3089.4372	9302.7506
1027	2648.7673	3092.8828	9314.4346
1028	2651.7793	3096.3289	9326.1213
1029	2654.7917	3099.7754	9337.8110
1030	2657.8046	3103.2223	9349.5038
1031	2660.8178	3106.6697	9361.1993
1032	2663.8315	3110.1174	9372.8977
1033	2666.8456	3113.5656	9384.5991
1034	2669.8601	3117.0142	9396.3033
1035	2672.8751	3120.4633	9408.0105
1036	2675.8904	3123.9127	9419.7206
1037	2678.9062	3127.3625	9431.4336
1038	2681.9224	3130.8128	9443.1494
1039	2684.9390	3134.2635	9454.8682
1040	2687.9560	3137.7147	9466.5897
1041	2690.9735	3141.1662	9478.3142
1042	2693.9914	3144.6181	9490.0416
1043	2697.0096	3148.0705	9501.7719
1044	2700.0284	3151.5233	9513.5050
1045	2703.0475	3154.9765	9525.2410
1046	2706.0670	3158.4301	9536.9799
1047	2709.0869	3161.8842	9548.7216
1048	2712.1073	3165.3386	9560.4662
1049	2715.1281	3168.7935	9572.2136
1050	2718.1493	3172.2487	9583.9640

TABLE 24. THE DIVERSITY OF SPECIES, $\bar{d}$, CHARACTERISTIC OF MacARTHUR'S MODEL FOR VARIOUS NUMBERS OF HYPOTHETICAL SPECIES, s'*

s'	$\bar{d}$	s'	$\bar{d}$	s'	$\bar{d}$	s'	$\bar{d}$
1	0.0000	51	5.0941	102	6.0792	205	7.0783
2	0.8113	52	5.1215	104	6.1069	210	7.1128
3	1.2997	53	5.1485	106	6.1341	215	7.1466
4	1.6556	54	5.1749	108	6.1608	220	7.1796
5	1.9374	55	5.2009	110	6.1870	225	7.2118
6	2.1712	56	5.2264	112	6.2128	230	7.2434
7	2.3714	57	5.2515	114	6.2380	235	7.2743
8	2.5465	58	5.2761	116	6.2629	240	7.3045
9	2.7022	59	5.3004	118	6.2873	245	7.3341
10	2.8425	60	5.3242	120	6.3113	250	7.3631
11	2.9701	61	5.3476	122	6.3350	255	7.3915
12	3.0872	62	5.3707	124	6.3582	260	7.4194
13	3.1954	63	5.3934	126	6.3811	265	7.4468
14	3.2960	64	5.4157	128	6.4036	270	7.4736
15	3.3899	65	5.4378	130	6.4258	275	7.5000
16	3.4780	66	5.4594	132	6.4476	280	7.5259
17	3.5611	67	5.4808	134	6.4691	285	7.5513
18	3.6395	68	5.5018	136	6.4903	290	7.5763
19	3.7139	69	5.5226	138	6.5112	295	7.6008
20	3.7846	70	5.5430	140	6.5318	300	7.6250
21	3.8520	71	5.5632	142	6.5521	310	7.6721
22	3.9163	72	5.5830	144	6.5721	320	7.7177
23	3.9779	73	5.6027	146	6.5919	330	7.7620
24	4.0369	74	5.6220	148	6.6114	340	7.8049
25	4.0937	75	5.6411	150	6.6306	350	7.8465
26	4.1482	76	5.6599	152	6.6495	360	7.8870
27	4.2008	77	5.6785	154	6.6683	370	7.9264
28	4.2515	78	5.6969	156	6.6867	380	7.9648
29	4.3004	79	5.7150	158	6.7050	390	8.0022
30	4.3478	80	5.7329	160	6.7230	400	8.0386
31	4.3936	81	5.7506	162	6.7408	410	8.0741
32	4.4381	82	5.7681	164	6.7584	420	8.1087
33	4.4812	83	5.7853	166	6.7757	430	8.1426
34	4.5230	84	5.8024	168	6.7929	440	8.1757
35	4.5637	85	5.8192	170	6.8099	450	8.2080
36	4.6032	86	5.8359	172	6.8266	460	8.2396
37	4.6417	87	5.8524	174	6.8432	470	8.2706
38	4.6792	88	5.8687	176	6.8596	480	8.3009
39	4.7157	89	5.8848	178	6.8758	490	8.3305
40	4.7513	90	5.9007	180	6.8918	500	8.3596
41	4.7861	91	5.9164	182	6.9076	550	8.4968
42	4.8200	92	5.9320	184	6.9233	600	8.6220
43	4.8532	93	5.9474	186	6.9388	650	8.7373
44	4.8856	94	5.9627	188	6.9541	700	8.8440
45	4.9173	95	5.9778	190	6.9693	750	8.9434
46	4.9483	96	5.9927	192	6.9843	800	9.0363
47	4.9787	97	6.0075	194	6.9992	850	9.1236
48	5.0084	98	6.0221	196	7.0139	900	9.2060
49	5.0375	99	6.0366	198	7.0284	950	9.2839
50	5.0661	100	6.0510	200	7.0429	1000	9.3578

*The data in this table are reproduced, with permission, from Lloyd and Ghelardi

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APPENDIX A

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
PORIFERA						
Anheteromeyenia						
ryderi	X		Yes		3	
Ephydatia						
fluviatilis	X		No	4		
muelleri	X		No		2	
Eunapius						
fragilis	X		Yes	4		
Heteromeyenia						
tubisperma		X	No		3	
Spongilla						
lacustris	X		No		3	
Trochospongilla						
horrida	X		No		3	
pennsylvanica	X		No		2	
BRYOZOA						
Fredericella						
sultana					2	
Hyalinella						
punctata	X		No		2	
Lophopodella						
carteri						1
Pectinatella						
magnifica		X				1
Plumatella						
casmiana		X			3	
emarginata		X		4		
repens	X			4		
Urnatella						
gracilis					3	
COELENTERATA						
Cordylophora						
lacustris					2	
Craspedacusta						
sowerbyi					2	
TURBELLARIA						
Cura						
foremanni					2	
Dugesia						
dorotocephala				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
tigrina				4		
Phagocata						
gracilis					2	
NEMERTEA						
Prostoma						
graecense					3	
ANNELIDA - POLYCHAETA						
SABELLIDAE						
Manayunkia						
speciosa					3	
ANNELIDA - OLIGOCHAETA						
NAIDIDAE						
Amphichaeta						
americana					2	
Chaetogaster						
diaphanus					2	
diastrophus					2	
Dero						
digitata					2	
nivea					3	
obtusa					3	
pectinata					2	
Nais						
barbata				4		
behningi					3	
bretscheri					3	
communis				4		
elinguis				5		
pardalis				4		
simplex					3	
variabilis				5		
Ophidonais						
serpentina				4		
Pristina						
aequiseta					3	
Slavina						
appendiculata					2	
Specaria						
josinae					2	
Stylaria						
fossularis					3	
lacustris					3	
Vejdovskyella						
comata						1
intermedia				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
TUBIFICIDAE						
Aulodrilus						
americanus					3	
limnobius					3	
pigueti					3	
pluriseta					3	
Bothrioneurum						
vejdovskyanum					2	
Branchiura						
sowerbyi				4		
Ilyodrilus						
templetoni					3	
Isochaetides						
curvisetosus					2	
Limnodrilus						
cervix				4		
claparedianus				4		
hoffmeisteri				5		
maumeensis				5		
udekemianus				5		
Potamothrix						
moldaviensis					3	
vejdovskyi					3	
Quistadrilus						
multisetosus				4		
Spirosperma						
carolinensis					3	
ferox					3	
multisetosus				4		
nikolskyi					2	
Tubifex						
tubifex	X			5		
ANNELIDA - HIRUDINEA						
ERPOBDELLIDAE						
Erpobdella						
parva				4		
punctata				4		
Mooreobdella						
microstoma				4		
HAEMOPIDAE						
Haemopis						
grandis					3	
marmorata					3	
GLOSSIPHONIIDAE						
Alboglossiphonia						
heteroclita					3	
Gloiobdella						
elongata				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Helobdella						
stagnalis				4		
triserialis					3	
Glossiphonia						
complanata	X			4		
Placobdella						
multilineata					2	
ornata					3	
papillifera					3	
parasitica					2	
PISCICOLIDAE						
Myzobdella						
lugubris					3	
Piscicola						
punctata					3	
HYDRACARIA						
Albia						
stationis						1
Arrenurus						
kenki					2	
planus					3	
serratus					2	
Bandakia						
anisitsipalpis						0
elongata						0
Euthyas						
truncata					2	
Frontipoda						
americana					2	
Forelia						
cooki					2	
Hydrachna						
conjecta						1
crenulata						1
magnisculata						1
miliaria						1
rotunda						1
stipata						1
Hydrodromia						
despiciens					2	
Hydryphantes						
tenuabilis					3	
Hygrobates						
fluviatilis				4		
longipalpis				4		
neooctoporus					2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Lebertia						
quinquemaculosa						1
Limnesia						
maculata					2	
undulata					2	
Neumania						
rotundra						1
Oxus						
connatus						1
Piona						
carnea						1
constricta					2	
pugilis						1
rotunda						1
Pirata						
insularis					2	
Sperchon						
crassipalpus						0
glandulosus				4		
Sperchonopsis						
verrucosa						1
Testudacarus						
minimus						1
Thyas						
barbigera					2	
bruzelii					2	
stolli					2	
Tiphys						
americanus					3	
simulans					3	
Unionicola						
formosa					3	
ARTHROPODA - CRUSTACEA						
ISOPODA						
Asellus						
attenuatus					3	
brevicaudatus				4		
communis					3	
intermedius					3	
militaris					3	
racovitzai					3	
Lirceus						
fontinalis		X			3	
lineatus						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
AMPHIPODA						
Crangonyx						
gracilis					3	
obliquus						1
pseudogracilis	X				3	
serratus					2	
Gammarus						
fasciatus					2	
lacustris					2	
minus					2	
pseudolimnaeus		X			2	
tigrinus					2	
Hyallela						
azteca					2	
Synurella						
chamberlaini					2	
DECAPODA						
Cambarus						
acuminatus					3	
asperimanus						1
bartonii			Yes		3	
exraneus						1
diogenes			Yes	4		
floridanus					2	
longirostris					3	
longulus			No			1
Orconectes						
immunis					2	
obscurus			Yes		2	
propinquus			No		2	
rusticus					3	
virilis						1
Palaemonetes						
exilipes				4		
kadiakensis					2	
paludosus					2	
Procambarus						
acutus				4		
clarkii					3	
MYSIDACEA						
Mysis						
relicta						1

POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
INSECTA - DIPTERA						
CHIRONOMIDAE						
Ablabesmyia						
aequifasciata				4		
americana					2	
annulata			Yes			1
aspera		X	Yes		2	
auriensis						1
basalis			Yes		2	
cinctipes			Yes		2	
hauberi			Yes			1
illinoensis						1
janta					3	
mallochi		X	No		2	
monilis		X	Yes		2	
parajanta			Yes		3	
peleensis					2	
philosphagnos			Yes		2	
rhamphe					2	
tarella					3	
Arctopelopia						
flavifrons			Yes			0
Boreochlus						
persimilis			Yes			0
Brillia						
flavifrons					2	
par		X				1
parva						0
Calopsectra						
confusa			Yes			1
dendyi			Yes		2	
gregarius				4		
neoflavella			Yes		2	
Cardiocladius						
obscura			Yes		2	
platypus			Yes			0
Chaetocladis						
atroviridis						0
ochreatus						0

POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Chironomus						
anthracinus					3	
atrella			Yes	4		
attenuatus	X		Yes	4		
carus					3	
crassicaudatus			Yes		3	
flavus					3	
fulvipilus				4		
paganus					3	
plumosus			Yes	5		
riparius		X	No	5		
staegeri			Yes		3	
stigmaterus					3	
tentans					3	
tuxis			Yes			1
Cladotanytarsus						
viridiventris			No		2	
Clinotanypus						
caliginosus						1
pinguis		X	Yes		3	
Coelotanypus						
concinnus			No	4		
scapularis					3	
tricolor					2	
Corynoneura						
scutellata						1
taris			Yes			1
Cricotopus						
absurdus						1
bicinctus	X		No		2	
exilis						1
fugax			Yes			0
politus						1
remus			No		2	
sylvestris			No		2	
tremulus					3	
tricinctus					2	
trifasciatus					3	
Cryptochironomus						
blarina			No		2	
fulvus					3	
digitatus					3	
nais					3	
ponderosus					3	
psittacinus						1
sorex						1

POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Cryptotendipes						
casuarius			Yes			1
darbyi					2	
emorsus			No		2	
Demeijerea						
atrimanus			Yes		2	
brachialis			No		2	
Demicryptochironomus						
vulneratus						1
Diamesa						
nivoriunda			Yes			1
spinacies			No			0
Dicrotendipes						
californicus					2	
fumidus		X	No			1
incurvus			Yes		3	
leucoscelis			Yes			1
lobus			Yes			1
modestus		X	No		3	
neomodestus					2	
nervosus		X	No		2	
Einfeldia						
austeni			No		3	
brunneipennis			No		3	
natchitocheae					3	
Endochironomus						
nigricans					3	
Epiococladius						
flavens			No			0
Eukiefferiella						
coerulescens						0
Glyptotendipes						
amplus					3	
barbipes				4		
lobiferus			No		3	
meridionalis			No	4		
paripes			No		3	
senilis						1
Goeldichironomus						
holoprasinus			No	5		
Guttipelopia						
currani			Yes			1

POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Harnishia						
amachaerus			Yes		2	
boydi			Yes		3	
collator					3	
curtilamellata					2	
edwardsi			No		2	
galeator			No		2	
tenuicaudata					2	
viridulus			No		2	
Heterotrissocladius						
marcidus						0
Hydrobaenus						
pilipes					2	
Kiefferulus						
dux					3	
Labrundinia						
becki			Yes			0
floridana						1
johannseni			Yes		2	
neopilosella			Yes			1
pilosella			Yes		2	
virescens					2	
Larsia						
lurida					2	
Lauterborniella						
agrayloides			No			0
varipennis			Yes		3	
Leptochironomus						
nigrovittatus					2	
Macropelopia						
decedens			Yes			1
Metriocnemus						
abdomino-flavatus			Yes	4		
hamatus			Yes			0
knabi			Yes	4		
lundbecki	X					1
Micropsectra						
deflecta						1
dives			Yes		2	
dubia			No			1
nigripila					3	
polita			No			0
Microtendipes						
pedellus		X	Yes			1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Monopelopia						
boliekae						0
tillandsia			Yes	4		
Nanocladius						
alternantherae			No		2	
balticus						1
distinctus					3	
minimus						1
parvulus						1
Natarsia						
fastuosa			No			0
Nilodorum						
devineyae					2	
Nilotanypus						
americanus			Yes		3	
Nilothauma						
babyi						1
bicornis					2	
Odontomesa						
fulva			No			0
Omisus						
pica			Yes			1
Orthocladius						
annectens			Yes		2	
obumbratus			Yes			1
Pagastiella						
orophila			Yes		3	
ostansa					2	
Parachironomus						
abortivus					3	
alatus			Yes		2	
carinatus			No		3	
directus			No		2	
hirtalatus					3	
loganae			Yes			1
monochromus			No		3	
pectinatellae					3	
potamogeti						1
schneideri			Yes		3	
sublettei					2	
tenuicaudatus					2	
Paracladopelma						
nais					3	
undine			Yes			1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Paralauterborniella						
elachista			No		3	
nigrohalteralis					3	
subcincta			No		2	
Paramerina						
anomala			Yes			0
smithae			No			0
Parametriocnemus						
lundbeckii						0
Paratendipes						
albimanus	X		No			1
subaequalis					2	
thermophilus			No			0
Parochlus						
kiefferi			No			0
Pedionomus						
beckae					2	
Pentaneura						
americana					2	
carneosa					2	
comosa			No			0
flavifrons				4		
inconspicua			Yes		2	
inculta					2	
melanops				4		
ornata			Yes		2	
Phaenopsectra						
profusa					2	
Polypedilum						
angulum					1	
apicatum			No			1
aviceps						1
braseniae			Yes			1
convictum					2	
digitifer			No		2	
fallax		X	Yes		3	
halterale		X	No		3	
illinoense	X		Yes		3	
labeculosum			No			0
laetum					3	
nubeculosum					2	
obtusum			Yes			1
scalaenum	X		Yes		2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
simulans			No		3	
sordens						1
trigonum			No		2	
tritum					3	
vibex						1
Procladius						
adumbratus			No		2	
bellus		X	No		3	
culiciformis			Yes		2	
denticulatus			No	4		
riparius			No		2	
Prodiamesa						
olivacea			Yes			0
Psectrocladius						
elatus					2	
julia					3	
niger					3	
vernalis			Yes		2	
Psectrotanypus						
dyari				4		
venustus			No			1
Pseudochironomus						
fulviventris					2	
julia					2	
richardsoni			No		2	
Psilotanypus						
bellus				4		
Rheocricotopus						
robacki					3	
Rheotanytarsus						
exiguus		X	Yes		3	
Robackia						
claviger					3	
demeijerei					3	
Sergentia						
coracina			No			0
Smittia						
aterrima			Yes			1
Stempellina						
johanseni					2	
Stenochironomus						
hilaris			No			1
macateei			No			1
Stictochironomus						
devinctus		X	Yes			0
varius			No		2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	Heavy Metals Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	Tolerance to Organic Wastes* Facultative	Tolerance to Organic Wastes* Intolerant
Tanypus						
carinatus			No		3	
clavatus			No		2	
grodhausi					3	
neopunctipennis					3	
parastellatus			No			1
punctipennis		X	No	4		
stellatus		X	No		3	
Tanytarsus						
buckleyi			No		2	
dissimilis						1
gracilentus						1
neoflavellus					3	
quadratus			No		2	
recens			No		2	
Thalassomyia						
bureni			No		2	
Thienemanniella						
xena						0
Thienemannimyia						
barberi			No			0
senata			Yes			0
Tribelos						
fuscicornis			Yes			1
jucundus		X	Yes			1
Trichocladius						
robacki						1
Xenochironomus						
rogersi			Yes			1
scopula		X	No		2	
taenionotus					2	
xenolabis		X	No		2	
Zavrelimyia						
carneosa						0
OTHER DIPTERA						
Anopheles						
crucians					3	
punctipennis					2	
Antocha						
saxicola			No			1
Atherix						
variegata		X			2	
Bezzia						
glabra				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Blepharicera						
tenuipes						0
Brachydeutera						
argentata				4		
Cnephia						
dacotensis						0
mutata					2	
pecuarum					2	
Chaoborus						
albatus					3	
americanus					2	
flavicans					2	
punctipennis					3	
Culex						
attratus					3	
erraticus					3	
pipiens				4		
restuans					3	
Eristalis						
aeneus				5		
bastardii				5		
brousii				5		
Mansonia						
titillans					3	
Metasyrphus						
americanus				4		
Odontomyia						
cincta					3	
Palpomyia						
tibialis					3	
Prosimulium						
fuscum					3	
gibsoni					3	
johannseni						1
magnum						1
mixtum					3	
mysticum						1
rhizophorum						1
Protoplasa						
fitchii					3	
Pseudolimnophila						
luteipennis						1
Psychoda						
alternata			Yes	5		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	Heavy Metals Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	Tolerance to Organic Wastes* Facultative	Tolerance to Organic Wastes* Intolerant
Simulium						
aurium				4		
clarkei					2	
corbis						1
croxtoni						1
decorum					2	
euryadminiculum						1
fibrinflatum					3	
jenningsi					2	
johannseni						0
latipes						1
luggeri					3	
meridionale						1
pictipes					2	
rugglesi					3	
tuberosum					2	
venustum					2	
verecundum					3	
vittatum					3	
Sphaeromais						
longipennis					3	
Stegopterna						
mutata					3	
Stilobezzia						
antennalis				4		
Stratiomys						
discalis				4		
meigenii				4		
Tabanus						
atratus					3	
benedictus				4		
giganteus						1
lineola				4		
stygius					2	
variegatus						1
Telmatoscopus						
albipunctatus				4		
Tipula						
abdominalis	X		Yes			1
caloptera						1
Toxorhynchites						
rutilus					3	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
INSECTA - TRICOPTERA						
Agarodes						
distinctum					2	
Agrypnia						
vestita						1
Amiocentrus						
aspilus			No			0
Anisocentropus						
pyraloides			Yes			0
Apatania						
incerta						0
Aphropsyche						
doringa						0
Arctopsyche						
grandis		X				1
irrorata			Yes			0
ladogensis						0
Asynarchus						
montanus					3	
Brachycentrus						
americanus			No			1
incanus						0
lateralis			No			0
numerosus						1
occidentalis						1
Ceraclea						
ancylus					2	
cancellata						0
diluta						1
flava			No			1
maculata						1
neffi						1
nepha					2	
punctata						0
slossonae					2	
tarsipunctata			No			1
transversa		X				1
Ceratopsyche						
alhedra					2	
alternans					2	
bronta					3	
bifida					3	
morosa						1
slossonae					2	
sparna						1
walkeri						1
vexa					2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Chimarra						
atterrima		X	Yes			1
feria						1
obscura					2	
perigua						1
socia						0
Culophila						
thoracica						0
Cyrnellus						
fraternus		X	No		3	
Diplectrona						
metaqui						0
modesta			Yes			1
Dolophilodes						
distinctus						0
Fattigia						
pele			Yes			1
Frenesia						
missa						0
Glyphopsyche						
irrorata						1
Goera						
calcarata						0
fuscula						0
stylata						0
Helicopsyche						
borealis		X	No		2	
Hesperophylax						
designatus					2	
Heteroplectron						
americanum						0
Hydatophylax						
argus						1
Hydropsyche						
aerata					2	
arinale					3	
betteni	X		Yes		3	
bidens					2	
cuanis					3	
demora					3	
depravata					3	
dicantha						1
frisoni		X	No		3	
incommoda					2	
leonardi						0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
orris		X	No		3	
phalerata						1
placoda					2	
scalaris						1
simulans		X	No		2	
venularis						1
Hydroptilia						
waubesiana					2	
Ironoquia						
punctatissima					2	
Leptocerus						
americanus						1
Leuchotrichia						
pictipes						1
Limnephilus						
rhombicus			No			1
submonilifer			No			1
Lype						
diversa			Yes			1
Macronemum						
carolina		X				0
zebratum					2	
Matrioptila						
jeanae						0
Micrasema						
kluane						1
rusticum						1
wataga						1
Molanna						
blenda			Yes			1
Mystacides						
sepulchralis	X		No			1
Nectopsyche						
albida						1
dorsalis					2	
exquisita						0
pavida					2	
Nemotaulius						
hostilis					2	
Neureclipsis						
crepuscularis		X	Yes			1
Oligostomis						
ocelligera						1
Onocosmoecus						
quadrinotatus						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Oropsyche						
howellae						0
Palaeagapetus						
celsus						0
Parapsyche						
apicalis						0
Phylocentropus						
placidus		X	Yes		2	
Potamyia						
flava		X	Yes		3	
Pseudogoera						
singularis						0
Pseudostenophylax						
uniformis						0
Psilotreta						
indecisa						0
Psychoglypha						
subborealis						0
Psychomyia						
flavida						1
Pycnopsyche						
gentilis			Yes		2	
guttifer	X		Yes		2	
lepida	X				2	
Rhyacophila						
acutilaba						0
amicis			Yes			0
atrata			Yes			0
brunnea						0
carolina			Yes		2	
carpenteri						0
fuscula						1
glaberrima			Yes		3	
invaria			Yes			1
ledra						1
lobifera					2	
melita						0
mycta						0
nigrita			Yes		2	
torva			Yes		2	
vibox						1
vulphipes						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Symphitopsyche						
bifida	X		No		3	
bronta					2	
macleodi						0
morosa					2	
riola					2	
sparna			Yes		3	
Trentonius						
distinctus					2	
Wormaldia						
moestus						0
INSECTA - EPHEMEROPTERA						
Ameletus						
lineatus						0
Ametropus						
albrighti						0
Arthroplea						
bipunctata			No			1
Attenella						
attenuata					2	
Baetis						
australis						0
bicaudatus			No			0
brunneicolor					2	
flavistriga					2	
frondalis					3	
hageni					3	
intercalaris					3	
longipalpus					3	
macdunnoughi					3	
propinquus					3	
pygmaeus					2	
spiethi			Yes			0
tricaudatus			No		2	
vagans			No		2	
Baetisca						
bajkovi			No		2	
carolina						0
escambiensis			No			0
gibbera			Yes			0
lacustris					3	
laurentina					2	
obesa			Yes			1
rogersi			Yes			0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	Heavy Metals Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	Tolerance to Organic Wastes* Facultative	Tolerance to Organic Wastes* Intolerant
Brachycercus						
lacustris	X					1
maculatus			Yes		2	
Caenis						
amica					3	
anceps						0
diminuta			Yes	4		
forcipata						1
hilaris			Yes			1
latipennis						0
simulans			No			1
Callibaetis						
coloradensis			No			0
floridanus			Yes	4		
pretiosus			No			0
Centroptilum						
viridocularis						0
Choroterpes						
basalis						1
hubbelli						1
Cloeon						
alamance						1
rubropictum			No			0
Dannella						
lita					2	
simplex						1
Dolania						
americana		X	Yes			0
Drunella						
cornutella						0
Epeorus						
albertae					2	
longimanus			No			1
vitreus			No			1
Ephemera						
blanda					2	
guttulata						1
simulans		X	No			1
varia						0
Ephemerella						
allegheniensis						0
aestiva						0
attenuata						0
aurivilli						0
berneri						0
bicolor	X		No		2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	Heavy Metals Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	Tolerance to Organic Wastes* Facultative	Tolerance to Organic Wastes* Intolerant
carolina					2	
catawba					2	
coloradensis			No			1
cornuta						1
coxalis						0
crenula						0
deficiens		X	No		3	
doddsi			No			1
dorothea						1
excrucians						1
flavilinea			No			0
frisoni						0
funeralis						1
grandis						0
hecuba			No		2	
inermis			No			0
invaria						1
lita	X					0
longicornis						0
needhami			No			1
rotunda						1
septentrionalis						0
serrata						1
serratoides						0
simplex		X	Yes			1
spiculosa						0
subvaria			No			1
temporalis					2	
teresa			No		3	
tibialis			No		2	
trilineata			Yes			1
versimilis						1
walkeri						1
wayah						0
Ephoron						
album			No			1
Eurylophella						
aestiva					3	
bicolor						1
funeralis						0
lutulenta					3	
temporalis					3	
Habrophlebia						
vibrans						0
Habrophlebiodes						
americana	X				2	
brunneipennis						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Heptagenia						
criddlei			No		2	
diabasia					2	
flavescens					2	
hebe			No		2	
lucidipennis					2	
maculipennis					2	
pulla			No			0
Heterocloeon						
curiosum						1
Hexagenia						
atrocaudata		X	No			0
bilineata			No		2	
limbata		X	No			1
munda			No		2	
rigida			No		2	
Homoeoneuria						
dolani	X					0
Isonychia						
bicolor					2	
pictipes						1
sadleri			No			0
Leptophlebia						
bradleyi			Yes			0
cupida			No			0
intermedia			Yes		2	
nebulosa			No	4		
nervosa					2	
Leucocuta						
hebe						1
Litobrancha						
recurvata					3	
Neoephemera						
purpurea						0
youngi		X	Yes			1
Nixe						
lucidipennis						1
Paraleptophlebia						
bicornuta			Yes		2	
bradleyi					3	
debilis			No			0
heteronea			No			1
mollis			No			1
praepedita			No			0
volitans			Yes		3	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	 Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	 Facultative	 Intolerant
Pentagenia						
vittigera		X	No		2	
Potamanthus						
distinctus						1
rufous						1
Pseudiron						
centralis					3	
Pseudocloeon						
carolina			No			1
dubium					2	
myrsum					2	
parvulum			No			1
punctiventris					2	
Rhithrogenia						
hageni			No		2	
impersonata						0
jejuna						0
pellucida						0
robusta			No			1
undulata						0
Serratella						
deficiens						1
sordida						1
Siphlonurus						
alternatus			No			0
Siphloplecton						
speciosum			Yes			0
Stenacron						
candidum						0
carolina						0
floridense			Yes			0
interpunctatum	X		Yes		2	
pallidum			No		2	
Stenonema						
annexum						0
ares			No			1
bipunctatum		X	No		2	
carlsoni						0
exiguum			Yes		3	
femoratum		X	No		3	
fuscum					2	
integrum			No		3	
ithaca						0
luteum						0
mediopunctatum						1
modestum						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
nepotellum			No		2	
pudicum						1
pulchellum			No		2	
quinquespinum			No			1
rubromaculatum			No			1
rubrum			Yes		2	
smithae			Yes		2	
terminatum			No		2	
tripunctatum		X	No		3	
vicarium	X		No			1
Tortopus						
incertus						1
Tricorythodes						
albilineatus			Yes			1
minutus			No		2	
INSECTA - PLECOPTERA						
Acroneuria						
abnormis		X	Yes		2	
arida			No			1
carolinensis						1
evoluta		X	No			1
georgiana						0
internata		X	No		2	
lycorias			Yes			1
perplexa					2	
ruralis	X		No			1
xanthenes						1
Agnetina						
capitata						1
Allocapnia						
granulata			No			0
nivicola			No			0
recta			No			0
rickeri			No			0
vivipara			No			1
Amphinemura						
delosa			Yes		2	
linda						0
Atoperla						
ephyre						0
Attaneuria						
ruralis						1
Brachyptera						
fasciata						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Clioperla						
clio						1
Diploperla						
duplicata			No			0
Eccoptura						
xanthenes						1
Hastaperla						
brevis			Yes			1
Hesperoperla						
pacifica			No		2	
Hydroperla						
crosbyi			No			0
Isogenoides						
frontalis						0
olivaceus						0
Isogenus						
bilobatus						1
decisus					2	
subvarians					2	
Isoperla						
bilineata			No		2	
clio			No			1
cotta						1
decepta			No			0
dicala						1
frisoni			Yes			1
fulva			No		2	
holochlora					2	
lata						1
marlynia			No			1
mohri			No			0
namata					2	
nana			No		2	
orata			No			1
richardsoni			No			1
signata			No			1
similis					2	
slossonae						1
transmarina			Yes			1
Leuctra						
ferruginea						0
sibleyi						0
tenella						0
tenuis						0
Nemocapnia						
carolina		X				0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals Tolerant	Heavy Metals Sensitive	Acid Tolerant	Tolerance to Organic Wastes* Tolerant	Tolerance to Organic Wastes* Facultative	Tolerance to Organic Wastes* Intolerant
Nemoura						
trispinosa						1
Neoperla						
clymene		X	No			1
stewarti						1
Oemopteryx						
glacialis						1
Paracapnia						
angulata						1
Paragnetina						
immarginata		X	No			1
media		X	No			1
Perlesta						
frisoni						1
placida			No		2	
Perlinella						
drymo		X				1
ephyre						1
Phasganophora						
capitata		X	No			1
Prostoia						
completa						1
similis						1
Shipsa						
rotunda						1
Soyedina						
vallicularia						0
Strophopteryx						
fasciata			No			1
Sweltsa						
mediana			Yes			0
Taeniopteryx						
burksi			No		2	
maura			Yes		2	
metequi					2	
nivalis		X	No			1
parvula						1
Zapada						
cinctipes			No			1
Zealeuctra						
claasseni			No			0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
INSECTA - ODONATA						
Aeshna						
umbrosa			No		2	
Amphiagrion						
saucium				5		
Anax						
junius			No		3	
Argia						
apicalis			No		3	
moesta	X		No	4		
translata			No		2	
Basiaeschna						
janata					3	
Boyeria						
grafiana					3	
vinosa	X		Yes		2	
Calopteryx						
aequabilis					3	
maculata					3	
Cannacria						
gravida					2	
Chromagrion						
conditum					2	
Coenagrion						
resolutum				4		
Cordulegaster						
erroneus					2	
fasciatus						1
maculatus					2	
sayi						0
Dromogomphus						
spinosus	X		No		2	
spoliatus			No			1
Didymops						
transversa					2	
Enallagma						
antennatum			No		3	
civile				4		
ebrium				4		
hageni				4		
signatum			Yes		2	
Epitheca						
cynosura					2	
princeps					2	
semiaquea						1

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Erythrodiplax						
berenice					2	
connata					2	
Gomphus						
externus			No		2	
pallidus					2	
plagiatus			No		2	
spiniceps			No		2	
vastus			No		2	
Hagenius						
brevistylus						1
Hetaerina						
americana					3	
titia			No			0
Hylogomphus						
brevis					2	
Ischnura						
posita			No		3	
verticalis			No	4		
Lanthus						
albistylus			No			1
parvulus					3	
Leucorrhinia						
intacta				4		
Libellula						
deplanata					2	
lydia					2	
pulchella				4		
Macromia						
georgiana						1
illinoiensis						1
taeniolata					2	
Neurocordulia						
molesta					2	
obsoleta			No			1
yamaskanensis						0
Pachydiplax						
longipennis			No		3	
Plathemis						
lydia				4		
Progomphus						
obscurus			No		3	
Stylogomphus						
albistylus						0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
INSECTA - NEUROPTERA						
Climacia						
areolaris		X	No			1
INSECTA - MEGALOPTERA						
Chauliodes						
pectinicornis					2	
rastricornis					2	
Corydalus						
cornutus		X	Yes		3	
Nigronia						
fasciatus						0
serricornis						1
Sialis						
infumata	X		Yes	4		
INSECTA - HEMIPTERA						
Belostoma						
fluminea	X		Yes	4		
Benacus						
griseus					2	
Callicorixa						
audeni			Yes		2	
Hydrometra						
martini				4		
Limnogonus						
hesione		X	No		3	
Merragata						
hebroides					3	
Mesovelia						
mulsanti					3	
Nepa						
apiculata						1
Rhagovelia						
obesa	X				3	
INSECTA - COLEOPTERA						
Anchytarsus						
bicolor						1
Ancyronyx						
variegatus			Yes		2	
Anodocheilus						
exiguus					2	
Bidessus						
fuscatus					2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Copelatus						
glyphicus					2	
Cybister						
fimbriolatus					3	
Derallus						
altus					2	
Dibolocelus						
ovatus					3	
Dineutus						
americanus				4		
Dubiraphia						
bivittata				4		
minima					3	
quadrinotata	X		Yes		3	
vittata	X				2	
Dytiscus						
hybridus					2	
Ectopria						
nervosa		X				0
Gonielmis						
dietrichi					2	
Graphoderus						
liberus					2	
Gyrinus						
floridensis				4		
Haliplus						
fasciatus					3	
Helichus						
lithophilus					3	
striatus					3	
Helochares						
maculicollis					2	
Hoperius						
planatus						1
Hydrochara						
obtusata						1
Hydrophilus						
triangularis						1
Hyogrotus						
farctus					2	
Laccobius						
agilis					2	
Laccophilus						
maculosus				4		
Laccornis						
difformis					2	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Macronychus						
glabratus	X		Yes		2	
Matus						
ovatus					2	
Microcylloepus						
pusillus						1
Optioservus						
fastiditus					2	
ovalis			No		2	
trivittatus						1
Oulimnius						
latiusculus						0
Pelonomus						
obscurus					2	
Peltodytes						
muticus					3	
sexmaculatus					3	
Promoresia						
elegans						0
tardella						0
Psephenus						
herricki		X	No			1
Ptilodactyla						
augustata						0
serricollis						0
Sperchopsis						
tesselatus					2	
Stenelmis						
crenata		X	Yes			1
decorata		X	No	4		
sexlineata		X	No		3	
Tropisternus						
dorsalis					3	
lateralis				4		
natator				4		
MOLLUSCA - GASTROPODA						
Amnicola						
emarginata						1
limosa			No			1
Aplexa						
hypnorum			No		2	
Bithynia						
tentaculata				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Campeloma						
decisum			Yes		3	
integrum					2	
rufum					2	
subsolidum					3	
Elimia						
livescens			No		3	
virginica			No	4		
Ferrissia						
fusca					3	
rivularis			No			1
tarda			No		3	
Fossaria						
modicella				4		
obrussa					3	
Gyraulus						
arcticus					3	
Helisoma						
anceps			No		3	
trivolvis			No	4		
Lioplax						
subcarinata						1
Lymnaea						
appressa						1
humilis					3	
ovata				5		
peregrina					3	
stagnalis			No		2	
Neoplanorbis						
carinatus						1
Physa						
fontinalis					2	
halei				4		
Physella						
acuta					2	
anatina				4		
cubensis				4		
gyrina				4		
heterostropha			No	4		
integra			No	4		
Planorbis						
trivolvis				4		
Planobula						
armigera				4		

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Pleurocera						
acuta			No		2	
lewisi					2	
Pseudosuccinea						
columella					3	
Radix						
auricularia					3	
Stagnicola						
caperata					3	
catascopium				4		
palustris			No		2	
Valvata						
bicarinata			No			1
piscinalis			No		2	
sincera			No		3	
tricarinata			No	4		
Viviparus						
contectoides						1
subpurpureus						1
MOLLUSCA - BIVALVIA						
Alasmidonta						
triangulata						0
undulata					2	
Amblema						
plicata			Yes		2	
Anodonta						
cataracta						1
gibbosus					3	
grandis					2	
imbecillus					2	
implicata						1
undulata					2	
Corbicula						
manilensis					3	
Cyclonaias						
tuberculata					2	
Elliptio						
complanata					2	
congaraea						1
icterina						1
shepardiana						0
waccamawensis						0

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

	Heavy Metals		Acid	Tolerance to Organic Wastes*		
Taxa	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Eupera						
cubensis					3	
Lampsilis						
cariosa						0
luteola					2	
ochracea						0
parvus					2	
teres					2	
Lasmigona						
complanata					2	
costata					2	
Leptodea						
fragilis						0
Margaritifera						
margaritifera						0
Musculium						
partumeium				4		
securis					2	
transversum					3	
Obliquaria						
reflexa						0
Pisidium						
abditum				4		
amnicum					2	
casertanum				4		
complanatum				4		
compressum				4		
crystalense					2	
fallax					2	
henslowanum					2	
idahoense				4		
subtruncatum					2	
Proptera						
alata						1
Quadrula						
lachrymosa					2	
pustulosa					2	
rubiginosa					2	
Rangia						
cuneata					3	

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

Taxa	Heavy Metals		Acid	Tolerance to Organic Wastes*		
	Tolerant	Sensitive	Tolerant	Tolerant	Facultative	Intolerant
Sphaerium						
corneum					3	
lilycashense					3	
notatum				4		
rhomboideum					3	
solidula						1
sulcatum					3	
stamineum					3	
striatinum					3	
transversum				4		
Strophitus						
edentulus					2	
Truncilla						
donaciformis						1
Uniomerus						
tetralasmus						1

*Ranking from 0 to 5 with 0 being the least tolerant.

References used in determining tolerances

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Hubbard, M.D. and W.L. Peters. 1978. Environmental requirements and pollution tolerance of Ephemeroptera. EPA-600/4/78-061. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH 45268.

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APPENDIX B

Hilsenhoff's Family Level Pollution Tolerance Values for Aquatic Arthropods[1]

Order	Family	Tolerance Value
Plecoptera	Capniidae	1
	Chloroperlidae	1
	Leuctridae	0
	Nemouridae	2
	Perlidae	1
	Perlodidae	2
	Pteronarcyidae	0
	Taeniopterygidae	2
Ephemeroptera	Baetidae	4
	Baetiscidae	3
	Caenidae	7
	Ephemerellidae	1
	Ephemeridae	4
	Heptageniidae	4
	Leptophlebiidae	2
	Metretopodidae	2
	Oligoneuriidae	2
	Polymitarcyidae	2
	Potomanthidae	4
	Siphlonuridae	7
	Tricorythidae	4
Odonata	Aeshnidae	3
	Calopterygidae	5
	Coenagrionidae	9
	Cordulegastridae	3
	Corduliidae	5
	Gomphidae	1
	Lestidae	9
	Libellulidae	9
	Macromiidae	3
Trichoptera	Brachycentridae	1
	Glossosomatidae	0
	Helicopsychidae	3
	Hydropsychidae	4
	Hydroptilidae	4
	Lepidostomatidae	1
	Leptoceridae	4

---

[1] From Hilsenhoff, 1988. Rapid field assessment of organic pollution with a family-level biotic index. J.N. Am. Benthol. Soc. 7(1):65-68.

Order	Family	Tolerance Value
Trichoptera (cont.)	Limnephilidae	4
	Molannidae	6
	Odontoceridae	0
	Philopotamidae	3
	Phryganeidae	4
	Polycentropodidae	6
	Psychomyiidae	2
	Rhyacophilidae	0
	Sericostomatidae	3
Megaloptera	Corydalidae	0
	Sialidae	4
Lepidoptera	Pyralidae	5
Coleoptera	Dryopidae	5
	Elmidae	4
	Psephenidae	4
Diptera	Athericidae	2
	Blephariceridae	0
	Ceratopogonidae	6
	Blood-red Chironomidae (Chironomini)	8
	Other (including pink) Chironomidae	6
	Dolochopodidae	4
	Empididae	6
	Ephydridae	6
	Psychodidae	10
	Simuliidae	6
	Muscidae	6
	Syrphidae	10
	Tabanidae	6
	Tipulidae	3
Amphipoda	Gammaridae	4
	Talitridae	8
Isopoda	Asellidae	8

APPENDIX C

EXAMPLES OF MACROINVERTEBRATE BENCH SHEETS

MACROINVERTEBRATE DATA SHEET

Type of Sampler____________________
Collection Depth___________________
Substrate Type____________________
Remarks________________________

Identification by__________________

Sample No.____________________
Date__________________________
Location_______________________

Station #_____________________
Collector_____________________

Enter Family and/or Genus and Species Name on Blank Line.

Organisms	No.	A.	I.
Diptera			
Chironomidae			
Other			
Trichoptera			
Plecoptera			
Ephemeroptera			
Odonata			
Hemiptera			

	No.	A.	I.
Coleoptera			
Neuroptera and Megaloptera			
Crustacea			
Oligochaeta			
Hirudinea			
Bivalvia			
Gastropoda			
Bryozoa			
Coelenterata			
Other			

A = Adult. I = Immature

Total No. Organisms__________________

Total No. Taxa______________________

MARINE MACROINVERTEBRATES

Name of water body____________________ Sample No.________________

Collected by________________________ Station No.________________

Sorted by__________________________ Date collected______________

Identified by________________________

Group*	Number of Orders	Number of Families	Number of Genera	Number of Species	Total Individuals
Porifera					
Hydrozoa					
Scyphozoa					
Anthozoa					
Ctenophora					
Turbellaria					
Rhynchocoela					
Echiura					
Priapulida					
Sipuncula					
Pogonophera					
Polychaeta					
Oligochaeta					
Hirudinea					
Monoplacophora					
Polyplacophora					
Aplacophora					
Bivalvia					
Gastropoda					
Scaphopoda					
Cephalopoda					
Merostomata					
Pycnogonida					
Ostracoda					
Cirripedia					
Leptostraca					
Stomatopoda					
Cumacea					
Tanaidacea					
Isopoda					
Amphipoda					
Decapoda					
Phoronida					
Bryozoa					
Entoprocta					
Brachiopoda					
Cinoidea					
Stelleroidea					
Echinoidea					
Holothuroidea					
Enteropneusta					
Pterobranchia					
Chaetognatha					
Urochordata					
Cephalochorsata					

*Use separate sheet for taxa names when identified beyond group.

APPENDIX D

EXAMPLE OF MACROINVERTEBRATE SUMMARY SHEET

MACROINVERTEBRATE LABORATORY - Summary of Data

Water Body________________________

Location________________________

Sampler________________________

Bottom Type________________________

Depth to Sampler________________________

Organisms:									
Diptera									
Chironomidae									
Other									
Trichoptera									
Plecoptera									
Ephemeroptera									
Odonata									
Anisoptera									
Zygoptera									
Neuroptera									
Hemiptera									
Coleoptera									
Lepidoptera									
Crustacea									
Amphipoda									
Isopoda									
Annelida									
Oligochaeta									
Hirudinea									
Turbellaria									
Mollusca									
Pelecypoda									
Gastropoda									
Bryozoa									
Coelenterata									
T. Individuals									
T. Species									

X = organisms present, not counted F - fragmented E - exuvia

Species Present:

SUMMARY OF MACROINVERTEBRATE DATA

STATION (LOCATION):

ORGANISM: Date										
Total Individuals										
Total Taxa										

APPENDIX E

LIST OF EQUIPMENT AND SUPPLIES

Listed below are equipment and supplies needed for the collection and analysis of macroinvertebrate samples. The data quality objectives and sampling and analysis methods should determine the type of equipment and supplies needed. The source numbers refer to the companies that are listed at the end of the table. Mention of these sources or products does not constitute endorsement by the U.S. Environmental Protection Agency.

Item	Unit	Source
Boat, flat bottom, 14-16 ft snatch-block meter wheel and trailer, 18 hp outboard motor. life jackets, other accessories	1	(7,15)
Boat crane kit and winch	1	(3,15)
Boat, inflatable with oar set	1	(1,15)
Cable fastening tools		(4,15)
Cable clamps, 1/8 "	25	
Nicro-press clamps, 1/8 "	100	
Nicro-press tool, 1/8 "	1	
Wire cutter, Felco	1	
Wire thimbles, 1/8 "	25	
Cable, 1/8 ", galvanized steel	1000 ft	(3,15)
Large capacity metal wash tub	1	
Sample wash bucket (sieve)	1	(8,14)
Core sampler, hand held	1	(3,8,14)
Box corer	1	(14)
K-B corer	1	(8)
Wide-barrel gravity corer	1	(14)
Phleger corer	1	(8,14)
Ballchek single or multiple corer	1	(8,14)
Ewing portable piston corer	1	(14)
Hardboard multiplate sampler	10	(3,8)
Ceramic multiplate sampler	10	(14)
Trawl net	1	(8)
Dredge	1	(3,8,14)
Rectangular box sediment sampler	1	(14)
Drift net, stream	6	(8,14)
Triple-net drift sampler	2	(14)
Stream bottom sampler, Surber type	2	(3,8,14)
Portable invertebrate box sampler	2	(13)
Stream-bed fauna sampler, Hess type	2	(14)
Hess stream bottom sampler	2	(8)
Grab sampler, Ponar	1	(3,8,14)

Wildco box corer	1	(8)
Grab sampler, Ekman	1	(3,8,14)
Grab sampler, Petersen	1	(3,8,14)
Grab sampler, Smith-McIntyre	1	(14)
Grab sampler, Van Veen	1	(14)
Grab sampler, Orange Peel	1	(14)
Grab sediment sampler, Shipek	1	(8)
Basket, bar B-Q, tumbler (#740-0035)	12	(9,11)
Sieves, US standard No. 30	2	(5)
Flow meter, mechanical	1	(3)
Mounting media, CMCP-9/9AF with stain	4 oz	No longer available
Mounting medium, CMCP-9	4 oz	(6)
Mounting medium, CMCP-10	4 oz	(6)
Fuchsin basic, C.I. dye	25 g	(6)
Mounting medium, Aquamount	4 oz	(12)
Refrigerated circulator	1	(5)
Water pump, epoxy-coated	2	(1)
Holding tank, constant temp	1	(10)
Balance, top-loading	1	(5)
Counter, 12-unit, 2X6	1	(3)
Counter, hand tally	2	(3)
Waders, with suspenders	1 pr	(1,15)
Boots, hip	1 pr	(1,15)
Raincoat	1	(3,15)
Magni-focuser, 2X	1	(5)
Microscope, field	1	(3)
Magnifier, illuminated + base	1	(3)
Magnifier, pocket, 5X, 10X, and 15X	1	(3)
Microscope, compound, with phase and bright-field, trinocular, 10X and 15X eyepieces, 4X, 10X, 20X, 45X and 100X objectives	1	(5)
Microscope, stereoscopic, with stand	1	(2)
Microscope slide dispenser	1	(1)
Microscope slides and cover slips, 12 and 15 mm circles	10 gross	(1)
Photographic system, photostar	1	(5)
Camera, photomicrographic, with 50 mm lens	1	(1,15)
Stirrer, magnetic	1	(5)
Aquarium, 10 gal., with cover, air pump and filter	1	(1,15)
Aquatic dip net, Model 412D	2	(3)
Jars, screw cap, specimen	5 dz	(1)
Bottles, Wide mouth, 32 oz	1 case	(1)
Specimen jars, wide mouth, 4 oz	48	(1)
Specimen jars, wide mouth, 6 oz	48	(1)
Vials, specimen, 1 oz	10 gross	(1)
Petri dish, ruled grid	4	(1)
Petri dish, compartmented	1 case	(1)
Watch glasses	10	(1)

Vacuum oven	1	(5)
Sounding lead and calibrated line	1	(3)
Forceps, watchman's, stainless	1 pr	(1)
Forceps, microdissection	2 pr	(1)
Dissecting set, basic	1	(1)
Water test kit, limnology	1	(1)
Thermometer, digital	1	(1)
Wash bottle, wide mouth, 500 mL	4	(1)
Wash bottle, polyethylene, 4 oz	2	(1)
Dropper bottle, polystop, 30 mL	2	(2)
Desiccator, polypropylene	1	(1)
Clip board with cover	2	(3,15)
Calculator, scientific	1	(3,15)
Marker, permanent, black	2	(3,15)
Pen set, slim pack, Koh-i-noor	1	(3,15)
Heavy paper tags with string	1000	(1,15)
Ice chest, insulated, 48 qt	2	(3,15)
Blue ice, soft pack	10	(3,15)
Plastic bags	100	(3,15)
Formalin, 10 percent	4 L	(2)
Ethyl alcohol	20 L	(2)
Trays, polypropylene, sorting	6	(5)

Sources of equipment and supplies:

1. Carolina Biological Supply Co.
   2700 York Rd.
   Burlington, NC 27215

2. Fisher Scientific
   50 Fadem Rd.
   Springfield, NJ 07081

3. Forestry Suppliers, Inc.
   205 West Rankin Street
   Jackson, MS 39284-8397

4. Industrial Rope Supply
   5250 River Rd.
   Cincinnati, OH 45233

5. Curtin Matheson Scientific, Inc.
   9999 Veterans Memorial Drive
   Houston, TX 77038-2499

6. Polyscience
   400 Valley Rd.
   Warrington, PA 18976

7. MonArk Boat Company
   Monticello, AK 71655

8. Wildlife Supply Company
301 Case Street
Saginaw, MI 48602

9. Tenaco
2007 NE 27th Ave.
Gainesville, FL 32609

10. Frigid Units, Inc.
3214 Sylvania Ave.
Toledo, OH 43613

11. W.C. Bradly Enterprises, Inc.
P.O. Box 1240
Columbus, GA 31993

12. Gallard-Schlesinger Chemical Mfg. Corp.
584 Mineola Avenue
Carle Place, NY 11514

13. Ellis-Rutter Associates
P.O. Box 401
Punta Gorda, FL 33950

14. Kahl Scientific Instrument Corp.
P.O. Box 1166
El Cajon, CA 92022-1166

15. Locally

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